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THE 37TH ANNUAL MEETING  
OF THE AMERICAN SOCIETY OF  
TROPICAL MEDICINE AND HYGIENE

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APR 27 1989  
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Omni Shoreham Hotel

Washington, DC

December 4 - December 8, 1988

December 30, 1988

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American Society of Tropical  
Medicine & Hygiene  
LSU Medical College  
P.O. Box 33932  
Shreveport, Louisiana 71130-3932

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## REGISTRATION

On Saturday 3 December 1988 the registration area (located on level one of the West Lobby) will be open from 2:00 PM to 6:00 PM. Sunday 4 December the registration area will be open from 8:00 AM to 6:00 PM. On Monday and Tuesday, registration hours will be from 7:30 AM until 4:00 PM, on Wednesday from 7:30 AM until Noon, on Thursday the registration desk will be open from 7:30 AM to 10:00 AM.

## SPONSORS

AMOCO  
Johnson and Johnson  
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Helminthological Society of Washington

## EXHIBITORS\*

Academia Book Exhibits  
Academic Press, Inc.  
American Type Culture Collection  
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U.S. Army Surgeon General's Office  
U.S. Naval Health Sciences Education and Training Command  
Wild Leitz U.S.A., Inc.

## TIME AND PLACE OF EXHIBITS

Place: Ambassador Room, third level of the West Conference Center, Omni Shoreham Hotel.

### Times:

Monday 5 December	12 Noon - 5:00 PM
Tuesday 6 December	8:00 AM - 5:00 PM
Wednesday 7 December	8:00 AM - 5:00 PM

\* As of September 12, 1988

SCHEDULE OF SPECIAL EVENTS, GROUP AND COMMITTEE MEETINGS

SUNDAY, DECEMBER 4

3:00 PM - 6:00 PM Pre-Meeting Workshop: Funding Opportunities for Research in Tropical Disease - H. Sheffield, Chairperson. Palladian.

8:00 AM - 5:00 PM ASTMH Council Meeting. Cabinet.

9:00 AM - 4:30 PM Pre-Meeting ASTMH and AAVP Workshop, Current Topics in Veterinary Parasitology of Significance to Human Medicine, Lecture Room A, Building A, Uniformed Services University of the Health Sciences (USUHS), Bethesda, MD.

1:00 PM - 4:00 PM SIRACA Subcommittee of ACAV. Embassy.

4:30 PM - 6:00 PM ACAV Council Meeting. Embassy.

5:00 PM - 6:30 PM ACME Council Meeting. Capitol.

7:00 PM - 9:00 PM Opening Reception. (Badges required). Blue Room.

MONDAY, DECEMBER 5

7:00 AM - 8:30 AM Editorial Board Meeting, American Journal of Tropical Medicine and Hygiene. Cabinet.

10:10 AM - 10:40 AM ASTMH Press Conference. Cabinet.

8:40 AM - 12:00 Noon ASTMH Opening Plenary Session. J.K. Frenkel and R.S. Nussenzweig presiding. Regency.

11:30 AM - 1:30 PM Congressional Briefing Luncheon. Cabinet.

1:30 PM - 6:30 PM Clinical Tropical Medicine Group Meeting. Diplomat.

5:00 PM - 6:00 PM ACME Business Meeting. Executive.

5:00 PM - 7:00 PM Portraits in Arbovirology: Viewing and Discussion. ASTMH Archives. Mezzanine of the Ambassador Room.

5:30 PM - 7:00 PM Workshop: Effective Advocacy. 1989 ASTMH Political Action Plan. Palladian.

TUESDAY, DECEMBER 6

7:00 AM Scientific Program Committee Meeting. Cabinet.

TUESDAY, DECEMBER 6 (Continued)

8:00 AM	Presidents' Breakfast. Forum.
8:00 AM - 10:30 AM	Poster Session I. Blue.
1:15 PM - 3:00 PM	Workshop: Late Breaking Advances in Molecular Biology. Palladian.
3:30 PM - 4:30 PM	ASTMH Presidential Address. Regency.
4:30 PM - 5:30 PM	ASTMH Annual Business Meeting. Regency.

WEDNESDAY, DECEMBER 7

8:00 AM - 10:30 AM	Poster Session II. Blue.
11:00 AM - 12:00 Noon	Craig Lecture. Regency.
12:00 AM - 1:30 PM	Paul C. Beaver Symposium. Palladian.
1:30 PM - 5:00 PM	ASTVM Annual Meeting - Ehrlichiosis Symposium. Executive.
7:00 PM	ASTMH Cocktail Party and Reception. Regency.
8:00 PM	ASTMH Banquet. Regency.

THURSDAY, DECEMBER 8

8:00 AM - 5:00 PM	ASTMH Council Meeting. Cabinet.
1:30 PM - 5:00 PM	Workshop: Cell-Mediated Immunity to Asexual Malaria Parasites. Ambassador.

## TOURS

Additional information on tours and other sightseeing activities in the Washington, D.C. area will be available in the Hospitality Suite located in the Committee Room on level one of the West Lobby. It will be open at 2:00 P.M. on Sunday 4 December, and will be open during the meeting from 8:00 A.M. - 5:00 P.M., December 5-8.

### DECEMBER 5

9:30 AM - 2:00 PM

A riding tour of historic Alexandria, your first stop will be at Christ Church, designed by James Wren and built in 1773. Then on to the Torpedo Factory, the cornerstone of Old Town cultural life. You may watch the artists at their craft. Everything is for sale, and commissions are welcome.

Lunch will be served at Gadsby's Tavern, George Washington's favorite Inn.

### DECEMBER 6

8:30 AM - 12:30 PM

Your first stop will be the Capitol, the most symbolic and representative building of our government. Next, visit the seat of our judicial system, the Supreme Court, the highest court in the land. The last stop will be the Library of Congress.

### DECEMBER 7

1:30 PM - 4:30 PM

A riding tour of Embassy Row. Washington's large, official diplomatic community. Also along this route you will see the Phillips Gallery, the Islamic Center, and the U.S. Naval Observatory. Then, take a tour of the Washington National Cathedral of St. Peter and St. Paul, which towers over the northwest section of Washington. Or if you would rather, your group could make a stop at the Woodrow Wilson House.

Finally, Anderson House. Built in 1902, this house is one of the few truly palatial residences remaining in Washington.

### AUDIOVISUAL FACILITIES

Slide projectors and screens will be available in the Director's Room on level one of the West Lobby beginning at 4:00 PM on Sunday, December 4 and continuing (from 8:00 AM - 5:00 PM) until 12 Noon on Thursday, December 7. These facilities are available for presenters to preview their slides. Investigators are responsible for previewing their slides and for placing their slides in carousels in advance of the scientific session.

Videotape facilities will also be available on request (please specify either beta or VHS and provide at least 24 hours notice) in the Director's Room.

### MICROSCOPES

A compound microscope will be available in the Director's Room on level one of the West Lobby beginning at 4:00 P.M. on Sunday, December 4 and continuing (from 8:00 A.M. - 5:00 P.M.) until 12 Noon on Thursday, December 7.

### ASTMH ARCHIVES

The ASTMH Archives will be on display on the Mezzanine of the Ambassador Room. The Society Archivist, Dr. Linda Brink, will be available from 1:30 PM to 6:00 PM on Monday, December 5 and from 8:30 AM to 6:00 PM on Tuesday December 6 and Wednesday December 7. There will be a special viewing of the Archive videotapes on outstanding arbovirologists, with informal discussion and refreshments on Monday, December 5 from 5:00 PM to 7:00 PM.

### HOSPITALITY SUITE

A Hospitality Suite for the spouses, friends and family of members attending the meeting will be located in the Committee Room on level one of the West Lobby. It will be open at 2:00 PM on Sunday 4 December, and will be open during the meeting from 8:00 AM - 5:00 PM, December 5-8.

### EMERGENCY CALLS AND MESSAGES

Emergency calls should be directed to (202)234-0700, extension 6791.

A message board will be available at the meeting Registration desk (level one of the West Lobby).

### EMPLOYMENT OPPORTUNITIES

Two bulletin boards will be available next to the Message Board.

### NOTICES

Badges must be worn to attend all functions.

Smoking will be permitted only where specifically authorized. This rule is in compliance with the Resolution on Smoking that was adopted at the ASTMH Annual Business Meeting on November 5, 1976. The cooperation and thoughtfulness of smokers is requested to minimize embarrassment and discomfort for all persons.

The time and/or location of all activities are subject to change. Change notices will be posted in the Registration Area.

### PROGRAM NOTES

The Symposia are intended to provide updates on subjects which have changed significantly in the last several years. They are designed especially for members who are not working actively in those areas, and often focus on work that has been published previously.

In contrast, the scheduled scientific sessions and the Late-Breaking Advances in Molecular Biology Workshop focus on recent unpublished results. Investigators interested in presenting in the Late-Breaker Molecular Biology session should contact Dr. Phil LoVerde (716-831-2459) or Dr. Thomas F. McCutchan (301-496-6149) within 3 weeks of the meeting or by noon on Monday, December 5 and come prepared to make a 10 minute oral presentation.

Oral presentations (in the regular sessions) should be 10 minutes or less in length unless scheduled otherwise, with 5 minutes for questions and discussion. Poster presentations are also based on recent unpublished work, but provide more time for informal discussion. The posters may be set up beginning at 7 PM the night before, but should be assembled by 8 AM. Authors should be in attendance from 8:00 AM to 10:30 AM at each poster session. Posters should be taken down by Noon on both Tuesday and Wednesday.

Suggestions for Changes in the meeting should be directed to the members of the Scientific Program Committee.

LOCAL COMMITTEE BUSINESS OFFICE AND INFORMATION CENTER

The Registration Area will be used as a business office for the Local Committee after the initial registrations have been completed on Monday December 5.

CONTINUING EDUCATION FOR PHYSICIANS

The American Society of Tropical Medicine and Hygiene is approved by the Accreditation Council on Continuing Medical Education to certify Category I CME credits for scientific portions of the Annual Meeting (up to 30 hours). Attendees who desire CME credit must pay a documentation fee of \$30 and must have paid their registration fee for the meeting. Each participant must return the CME Registration Form and the Attendance and Evaluation Form by the end of the meeting. Certificates based on the attendance recorded on the Attendance and Evaluation Form will be mailed to participants within one month of the meeting.

ABBREVIATED SCHEDULE OF CONCURRENT MEETING

HEALTH CARE FOR DISPLACED PERSONS AND REFUGEES: AN INTERNATIONAL SYMPOSIUM\*

Sponsored by Georgetown University Medical Center  
Sheraton Hotel, Washington, D.C.  
December 5-7, 1988

MONDAY, DECEMBER 5

Time

9:00 AM - 11:30 AM PLENARY SESSION ON HEALTH CARE OVERSEAS:

Major Medical Relief Efforts of the 1980's. A. Ali-Salad\*, G. Bertolaso, L. Clark, F. Cuny, P. Hakewill\*, B. Harrell-Bond\*, R. Kimura, J. Kronenberger, A.J. Rangaraj, L. Rosenblatt, S. Simmonds and M. Toole

CONCURRENT SCIENTIFIC PANELS AND WORKSHOPS ON HEALTH CARE OVERSEAS

Program A: Progress in Disease and Therapy

1:30 PM - 3:00 PM Controversies in Acute Respiratory Infections:  
E. Elias, S. Gove, P. Hopewell, and F. Luelmo

3:30 PM - 5:00 PM Advances in Diarrheal Diseases:  
A. Colon, R. Guerrant, R. Waldman and C. Weikel

Program B: Workshops on Health Care Delivery

1:30 PM - 3:00 PM I. Organization of Health Care Overseas (Part I):  
G. Bertolaso, O. Elo\*, M. Gabaudan, M. Lechat\*, A. Moren\*, C. Munoz\*, R. Russbach, H. Siem and M. Toole  
II. Mental Health Care Amidst Transcultural Differences.  
R. Cravens  
III. Essential Drugs and Medicines  
C. Albert\* and B. Snell\*  
IV. Internally Displaced Refugees (Angola, Ethiopia, Mozambique, Sri Lanka) Pending

3:30 PM - 5:00 PM I. Organization of Health Care Overseas (Part II):  
O. Elo\*, M. Gabaudan, M. Lechat\*, A. Miozzo, A. Moren\*, C. Munoz\*, W. Reich, H. Siem, S. Simmonds, M. Tailhades and M. Toole  
II. Indigenous Health (Ethnomedical) Practices:  
M. Nations and P. O'Connor\*  
III. Medical Training in the Refugee Setting:  
R. Kimura, R. Lee and R. Russbach  
IV. Maternal and Child Health  
M. Dualeh\*, S. Gove, R. Murphy\* and P. Torre\*

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\* - Persons registered for the ASTMH meeting may attend these sessions free of charge with their badge.

HEALTH CARE FOR DISPLACED PERSONS AND REFUGEES: AN INTERNATIONAL SYMPOSIUM\*

Sponsored by Georgetown University Medical Center  
Sheraton Hotel, Washington, D.C.  
December 5-7, 1988

TUESDAY, DECEMBER 6

9:00 AM - 11:30 AM PLENARY SESSION ON DEVELOPMENTS AND CONTROVERSIES IN  
NUTRITION  
A. Berry, B. Farah\*, P. Nieburg and J. Seaman

CONCURRENT PANEL DISCUSSIONS ON HEALTH CARE OVERSEAS AND AFTER RESETTLEMENT

Program A: Progress in Disease and Therapy

1:30 PM - 3:00 PM AIDS and the HIV's Among Refugees:  
D. Armstrong, D. Krumm, H. Masur and G. Slutkin

3:30 PM - 5:00 PM Developments in Viral Hepatitis:  
E. Tabor, M. Kane and V. Villarejos\*

Program B: Workshops on Health Care for Resettled  
Refugees and Expatriate Staff

1:30 PM - 3:00 PM I. Mental Health Care Amidst Transcultural Differences  
R. Cravens, P. DeLay and N. Tashima

II. Public Health Issues Among Resettled Ethnic Groups  
C. Gretenhart, J. Luoto and E. Perez-Stable

3:30 PM - 5:00 PM I. The Resettlement Experience from the Viewpoint of  
the Refugee

P. DeLay, C. Gretenhart and E. Perez-Stable  
II. Health Care of Expatriate Staff  
S. Kreider

CONCURRENT PANEL DISCUSSIONS ON HEALTH CARE OVERSEAS AND AFTER RESETTLEMENT

8:00 PM - 9:30 PM I. Developments in Malaria:  
C.C. Campbell, B. Doberstyn\* and R. Steketee  
II. Evaluating the Role of New Antimicrobials and  
Vaccines  
P. Pierce, D. Shepard and C. Wallace  
III. Controversies in Tuberculosis.  
F. Gordin, P. Hopewell, F. Luelmo, H. Rieder\* and G.  
Slutkin

WEDNESDAY, DECEMBER 7

9:00 AM - 11:30 AM PLENARY SESSION ON PRIORITIES IN REFUGEE HEALTH

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\* - Persons registered for the ASTMH meeting may attend these sessions free of charge with their badge.

SUMMARY OF THE ASTMH SCIENTIFIC PROGRAM

SUNDAY, DECEMBER 4TH

3:00 PM - 6:00 PM	Pre-Meeting Workshop: Funding Opportunities for Research in Tropical Disease - H. Sheffield, Chairperson. Ambassador.
8:00 AM - 5:00 PM	ASTMH Council Meeting. Cabinet.
1:00 PM - 4:00 PM	SIRACA Subcommittee of ACAV. Embassy.
4:30 PM - 6:00 PM	ACAV Council Meeting. Embassy.
5:00 PM - 6:30 PM	ACME Council Meeting. Capitol.
6:00 PM - 9:00 PM	ASTMH Opening Reception (Badges required). Blue.

MONDAY, DECEMBER 5TH

7:00 AM	Editorial Board Meeting, American Journal of Tropical Medicine and Hygiene. Cabinet.
8:40 AM - 12:00 Noon	ASTMH Opening Plenary Session. J.K. Frenkel and R.S. Nussenzweig presiding. Regency.
10:10 AM - 10:40 AM	Coffee Break and Press Conference.
12:00 Noon - 1:30 PM	Lunch Break
1:30 PM - 6:30 PM	Clinical Tropical Medicine Group Meeting. Diplomat.
1:15 PM - 5:00 PM	Symposium: Malarial Immunity in Mice and Humans. Palladian.
1:30 PM - 5:00 PM	Scientific Session A: Arbovirology - Arboviral Entomology. Regency.
1:30 PM - 5:00 PM	Scientific Session B: Filariasis - Epidemiology and Immunology. Executive.
1:30 PM - 5:15 PM	Scientific Session C: Antigens of Leishmania and Trypanosomes. Congressional.
3:00 PM - 3:30 PM	Coffee Break
5:00 PM - 6:00 PM	ACME Business Meeting. Executive.

SUMMARY OF THE ASTMH SCIENTIFIC PROGRAM (Continued)

MONDAY, DECEMBER 5 (Continued)

5:30 PM - 7:00 PM Special Session: Effective Advocacy Workshop. Palladian.

TUESDAY, DECEMBER 6TH

7:00 AM Presidents' Meeting and Breakfast. Cabinet.

7:00 AM Scientific Program Committee Meeting. Forum.

8:00 AM - 10:30 AM Poster Session I with Continental Breakfast. Blue.

10:00 AM - 12:00 Noon Symposium: The Water and Sanitation Decade (1981-1990): Its Impact on Tropical Diseases. Regency.

10:00 AM - 12:00 Noon Scientific Session D: Arbovirology - Epidemiology and Pathogenesis. Palladian.

10:00 AM - 12:00 Noon Scientific Session E: Filariasis - Biochemistry and Molecular Biology. Diplomat.

10:00 AM - 12:00 Noon Scientific Session F: Biochemistry and Molecular Biology of Kinetoplastida. Empire.

10:00 AM - 12:00 Noon Scientific Session G: Malaria - Biology and Immunopathology. Executive.

10:00 AM - 12:00 Noon Scientific Session H: Malaria - Epidemiology. Congressional.

12:00 Noon - 1:30 PM Lunch Break.

1:15 PM - 3:15 PM Scientific Session I: Schistosomiasis - General. Regency.

1:15 PM - 3:00 PM Scientific Session J: Workshop: Late Breaking Advances in Molecular Biology. Palladian.

1:30 PM - 3:00 PM Scientific Session K: Arbovirology - Diagnosis and Taxonomy. Diplomat.

SUMMARY OF THE ASTMH SCIENTIFIC PROGRAM (Continued)

TUESDAY, DECEMBER 6 (Continued)

1:30 PM - 3:00 PM	Scientific Session L: Malaria - Exoerythrocytic Antigens. Empire.
1:15 PM - 3:15 PM	Scientific Session M: Tropical Veterinary Medicine. Executive.
1:30 PM - 3:00 PM	Scientific Session N: Parasitic Entomology. Congressional.
3:00 PM - 3:30 PM	Coffee Break
3:30 PM - 4:30 PM	ASTMH Presidential Address - Jacob K. Frenkel. Regency.
4:30 PM - 5:30 PM	ASTMH Annual Business Meeting. Regency.

WEDNESDAY, DECEMBER 7TH

8:00 AM - 10:30 AM	Poster Session II with Continental Breakfast. Blue.
10:30 AM - 11:00 AM	Coffee Break
11:00 AM - 12:00 Noon	Craig Lecture: Duane Gubler. Regency.
12:00 Noon - 1:30 PM	Lunch Break.
1:30 PM - 5:00 PM	Special Session: Paul C. Beaver Symposium. Palladian.
1:30 PM - 5:30 PM	29th Annual Open Meeting of American Committee for Arthropod-Borne Viruses (ACAV). Diplomat.
1:30 PM - 5:00 PM	Scientific Session O: Malaria - Chemotherapy. Empire.
1:30 PM - 5:30 PM	ASTVM Annual Meeting: Ehrlichiosis Symposium. Executive.
1:30 PM - 5:00 PM	Scientific Session P: Malaria - Erythrocytic Antigens and Immunology. Congressional.
7:00 PM	ASTMH Cocktail Party and Reception. Regency.
8:00 PM	ASTMH Banquet. Regency.

SUMMARY OF THE ASTMH SCIENTIFIC PROGRAM (Continued)

THURSDAY, DECEMBER 8TH

7:00 AM	ASTMH Council Meeting. Cabinet.
8:00 AM - 11:00 AM	Scientific Session Q: Arbovirology - Molecular Biology and Vaccine Development. Ambassador.
11:00 AM - 12:00 Noon	Tropical Medicine Commemorative Fund Lecture: Progress in the Prevention and Treatment of Argentine Hemorrhagic Fever. J.I. Maiztegui. Ambassador.
8:15 AM - 12:00 Noon	Scientific Session R: Clinical Tropical Medicine. Diplomat.
8:15 AM - 12:00 Noon	Scientific Session S: Malaria - Sporozoites. Palladian.
8:45 AM - 12:00 Noon	Scientific Session T: Amebiasis and Giardiasis. Executive.
8:15 AM - 12:00 Noon	Scientific Session U: Filariasis - Surface Antigens. Congressional.
12:00 Noon - 1:30 PM	Lunch Break
1:30 PM - 5:00 PM	Workshop: Cell-Mediated Immunity to Asexual Malaria Parasites. Ambassador.
1:15 PM - 5:00 PM	Annual Meeting of the American Committee on Medical Entomology (ACME). Diplomat.
1:30 PM - 5:00 PM	Scientific Session V: Retroviral Infections and Epidemiology. Palladian.
1:30 PM - 5:00 PM	Scientific Session W: Schistosomiasis - Immunology. Executive.

DETAILED SCIENTIFIC PROGRAM

ASTMH OPENING PLENARY SESSION\*

MONDAY MORNING - DECEMBER 5

8:40 AM - 12:00 Noon

Regency

Chairpersons: J.K. Frenkel and R.S. Nussenzweig

Time    Abst

- 8:40                    WELCOME. J.K. Frenkel. President, ASTMH.
- 8:50            1        ANTIGEN PRESENTATION. Antonio Lanzavecchia. Basel Institute  
for Immunology, Basel, SWITZERLAND.
- 9:30            2        T CELL RECOGNITION: APPLICATIONS TO MALARIA AND AIDS. Jay A.  
Berzofsky. National Cancer Institute, National Institutes of  
Health, Bethesda, MD.
- 10:10                    Coffee Break
- 10:40            3        REGULATION OF IMMUNOGLOBULIN EXPRESSION BY LYMPHOKINES.  
William E. Paul. Chief, Laboratory of Immunology, National  
Institutes of Health, Bethesda, MD.
- 11:20            4        THE ACTIVATED MACROPHAGE AND PROTOZOAL INFECTION. Henry W.  
Murray. Professor of Medicine and Head, Division of  
Infectious Diseases, Cornell University Medical College, New  
York, NY.

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\* The ASTMH Opening Plenary Session is supported by the Agency for  
International Development Malaria Immunity and Vaccine Research Program.

MONDAY MORNING - DECEMBER 5

PRESS CONFERENCE\*

10:10 AM - 10:40 AM

Cabinet

Chairperson: J.K. Frenkel

DEFICIENCIES IN THE UNITED STATES' CAPACITY TO ADDRESS  
TROPICAL INFECTIOUS DISEASE PROBLEMS. K.M. Johnson, Former  
President, ASTMH.

ASTMH PLAN OF ACTION. S.F. Kuvin. Member, ASTMH Committee  
for Public Affairs.

MONDAY NOON - DECEMBER 5

CONGRESSIONAL BRIEFING LUNCHEON\*

12:00 Noon - 1:30 PM

Cabinet

Chairperson: J.K. Frenkel

Time    Abst

IMPACT OF TROPICAL MEDICINE PROBLEMS ON NORTH AMERICA. M.S.  
Wolfe, J.S. Keystone, and P.K. Russell. Department of State,  
Washington, DC; University of Toronto, Ontario, CANADA; and  
U.S. Army Medical Research and Development Command, Ft.  
Detrick, MD.

ASTMH Committee for Public Affairs and Legislative Task Force

A. Buck	S.M. Kuvin
J.A. Cook	P.K. Russell
J.R. David	S.R. Sagebiel
D.R. Hopkins	R.E. Shope
L. Jacobs	J. Smith
K.M. Johnson	G.D. Wallace
T.H. Weller	

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\* These sessions are intended for members of the media and Congress. The ASTMH Position Paper on Deficiencies in the United States' Capacity to Address Tropical Infectious Disease Problems and the Plan of Action will be provided to members at registration and will also be presented in detail at the Monday afternoon Workshop on Effective Advocacy in Palladian from 5:30 PM to 7:00 PM.

MONDAY AFTERNOON - DECEMBER 5

CLINICAL TROPICAL MEDICINE GROUP MEETING

1:30 PM - 6:30 PM

Diplomat

Chairpersons: F.J. Bia and M. Wittner

Time    Abst

- 1:30        5        CPC: INCONTINENCE AND RIGHT LEG PARESIS IN A 37 YEAR OLD MAN FROM GUATEMALA. DISCUSSANT: M. Barry. Yale University School of Medicine, New Haven, CT. PATHOLOGIST: M. Wittner. Albert Einstein College of Medicine, New York, NY.
- 2:15        6        UPDATE ON THE DIAGNOSIS AND TREATMENT OF LARVAL TAPEWORM DISEASE. P.M. Schantz. Centers for Disease Control, Atlanta, GA.
- 3:00               COFFEE BREAK
- 3:20        7        DIAGNOSTIC DILEMMAS: PROVOCATIVE CASES IN TROPICAL MEDICINE. J.S. Keystone, University of Toronto, Ontario, CANADA.
- 3:40        8        UPDATE ON ANTIMALARIAL CHEMOPROPHYLAXIS: DOXYCYCLINE, PROGUANIL AND FANSIDAR. C.C. Campbell and H.O. Lobe1. Malaria Branch, Centers for Disease Control, Atlanta, GA.
- 4:10               BUSINESS MEETING.  
Chairperson: M.S. Wolfe. Department of State, Washington, D.C.

MONDAY AFTERNOON, DECEMBER 5

SYMPOSIUM: MALARIAL IMMUNITY IN MICE AND HUMANS\*

1:15 PM - 5:00 PM

Palladian

Chairpersons: M.M. Stevenson and D.W. Taylor

<u>Time</u>	<u>Abst</u>	
1:15		OPENING COMMENTS. M.M. Stevenson.
1:20	9	HUMORAL IMMUNE RESPONSES TO MALARIAL PARASITES. D.W. Taylor, Georgetown University, Washington, DC.
1:45	10	ROLE OF CELL-MEDIATED IMMUNITY IN RESISTANCE TO MALARIA. J. Melancon-Kaplan and W.P. Weidanz. Hahnemann University School of Medicine, Philadelphia, PA.
2:10	11	CELL-MEDIATED IMMUNITY AND ITS ROLE IN PROTECTION. J.H.L. Playfair, K.R. Jones and J. Taverne. Middlesex Hospital Medical School, London, ENGLAND.
2:35	12	THE ROLE OF MACROPHAGES IN RESISTANCE TO MALARIA. H.L. Shear. New York University Medical Center, New York, NY.
3:00		COFFEE BREAK
3:30	13	MALARIA CRISIS FORMS, INTRAERYTHROCYTIC DEVELOPMENT DERANGEMENT. J.B. Jensen. Michigan State University, East Lansing, MI.
3:55	14	RELATIONSHIPS BETWEEN INFLAMMATION AND IMMUNOPATHOLOGY OF MALARIA. I.A. Clark and G. Chaudhri. Australian National University, Canberra, AUSTRALIA.
4:20	15	GENETIC CONTROL OF HOST RESISTANCE. M.M. Stevenson. McGill University and Montreal General Hospital Research Institute, Montreal, Quebec, CANADA.
4:45		CLOSING COMMENTS.

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\* This Symposium is supported by the Agency for International Development Malaria Immunity and Vaccine Research Program.

MONDAY AFTERNOON - DECEMBER 5

SCIENTIFIC SESSION A: ARBOVIROLOGY - ARBOVIRAL ENTOMOLOGY

1:30 PM - 5:00 PM

Regency

Chairpersons: P.A. Nuttall and W.K. Reisen

<u>Time</u>	<u>Abst</u>	
1:30	16	TRANSSTADIAL AND HORIZONTAL TRANSMISSION OF RIFT VALLEY FEVER VIRUS IN THE TICK <u>HYALOMMA TRUNCATUM</u> K.J. Linthicum,* T.M. Logan, C.L. Bailey, D.J. Dohm and J.R. Moulton. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.
1:45	17	CRIMEAN-CONGO HEMORRHAGIC FEVER IN SENEGAL: INFECTION RATES AND EPIDEMIOLOGIC ASSOCIATIONS OF TICK VECTORS AND VERTEBRATE HOSTS. M.L. Wilson,* B. LeGuanno, J.L. Camicas, J.P. Cornet, J.F. Saluzzo, J.P. Gonzalez and J.P. Digoutte. Institute Pasteur, Dakar, SENEGAL; Department of Tropical Public Health, Harvard University, Boston, MA: ORSTOM, L'Institut Pasteur, Disease Assessment Division, United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.
2:00	18	TISSUE TROPISMS AND REPLICATION STRATEGY OF DUGBE VIRUS (NAIROVIRUS, BUNYA VIRIDAE) IN <u>AMBLYOMMA VARIEGATUM</u> TICKS. G.M. Steele* and P.A. Nuttall. NERC Institute of Virology, Mansfield Road, Oxford, UNITED KINGDOM.
2:15	19	ANATOMICAL BASIS OF THOGOTO VIRAL INTERFERENCE IN THE TICK VECTOR, <u>RHIPICEPHALUS APPENDICULATUS</u> . L.D. Jones,* C.R. Davies, B.M. Green and P.A. Nuttall. N.E.R.C. Institute of Virology, Mansfield Road, Oxford, UNITED KINGDOM.
2:30	20	TRANSOVARIAN TRANSMISSION OF AFRICAN SWINE FEVER VIRUS BY <u>ORNITHODOROS MOUBATA</u> . R.G. Endris.* USDA-ARS, Plum Island Animal Disease Center, Greenport, NY.
2:45	21	DEVELOPMENT OF CONGENIC MOSQUITO LINES FOR THE STUDY OF MODULATION OF WESTERN EQUINE ENCEPHALITIS VIRUS. L.D. Kramer,* J.L. Hardy and S.B. Presser. University of California, Berkeley, CA.
3:00		COFFEE BREAK
3:30	22	INFLUENCE OF RIFT VALLEY FEVER VIRAL INFECTION ON THE DAILY SURVIVORSHIP OF <u>AEDES MCINTOSHI</u> AND <u>AEDES FOWLERI</u> MOSQUITOES. L.A. Patrican,* T.M. Logan and C.L. Bailey. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.
3:45	23	EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE ABILITY OF MOSQUITOES TO TRANSMIT OCKELBO VIRUS. J.O. Lundstrom* and M.J. Turell. Statens Bakteriologiska Laboratorium, Stockholm, SWEDEN; United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

- 4:00 24 EFFECT OF THE DEVELOPMENTAL STAGE AT INFECTION ON THE ABILITY OF ANOPHELES ALBIMANUS TO TRANSMIT RIFT VALLEY FEVER VIRUS. M.J. Turell.\* United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.
- 4:15 25 HOST-ADAPTIVE MODIFICATION OF LA CROSSE VIRUS: PRELIMINARY STUDIES. G.V. Ludwig,\* J.E. Osorio, B.M. Christensen and T.M. Yuill. University of Wisconsin at Madison, WI.
- 4:30 26 ORBIVIRUSES REPLICATE IN CELLS FROM CULICOIDES VARIIPENNIS. S.J. Wechsler\* and W.C. Wilson. United States Department of Agriculture, Agricultural Research Service, Arthropod-borne Animal Diseases Research Laboratory, Laramie, WY.
- 4:45 27 MOSQUITO ECOLOGY IN SUBURBAN COMMUNITIES IN THE GREATER LOS ANGELES AREA OF CALIFORNIA, USA. W.K. Reisen\* and R.P. Meyer. School of Public Health, University of California, Berkeley, CA.

MONDAY AFTERNOON - DECEMBER 5

SCIENTIFIC SESSION B: FILARIASIS - EPIDEMIOLOGY AND IMMUNOLOGY

1:30 PM - 5:00 PM

Executive

Chairpersons: P.J. Lammie and T.B. Nutman

- | <u>Time</u> | <u>Abst</u> |                                                                                                                                                                                                                                                                                                                                                                                                         |
|-------------|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1:30        | 28          | KNOWLEDGE, ATTITUDES AND PERCEPTIONS (KAP) OF ONCHOCERCIASIS: A SURVEY AMONG RESIDENTS IN SOME ENDEMIC AREAS IN GUATEMALA. F. Richards,* R.E. Klein and C. Gonzalez-Peralta. Medical Entomology Research and Training Unit/Guatemala, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.                                                                                         |
| 1:45        | 29          | COMPARATIVE DENSITIES OF <u>WUCHERERIA BANCROFTI</u> MICROFILARIA IN PAIRED SAMPLES OF CAPILLARY AND VENOUS BLOOD. M.L. Eberhard,* J.M. Roberts, P.J. Lammie and R.C. Lowrie, Jr. Centers for Disease Control, Atlanta, GA; Louisiana State University Medical Center, New Orleans, LA; Delta Regional Primate Research Center, Covington, LA.                                                          |
| 2:00        | 30          | IMMUNOLOGIC AND PARASITOLOGIC CHARACTERIZATION OF BANCROFTIAN FILARIASIS IN A HAITIAN PEDIATRIC POPULATION. W.L. Hitch,* P.J. Lammie, M.L. Eberhard and R.C. Lowrie, Jr. Louisiana State University Medical Center of New Orleans, LA; Centers for Disease Control, Atlanta, GA; Tulane University, Delta Regional Primate Center, Covington, LA; the ICIDR Program Tulane University, New Orleans, LA. |
| 2:15        | 31          | AN <u>IN VITRO</u> TECHNIQUE TO DESCRIMINATE TRANSMITTING MOSQUITOES INFECTED WITH <u>WUCHERERIA BANCROFTI</u> . A.N. Hassan, I.S. Abd El Azim and A.M. Gad.* Research and Training Center on Vectors of Diseases, Ain Shams University, Cairo, EGYPT.                                                                                                                                                  |

- 2:30 32 EFFECTIVENESS OF PIRIMIPHOS-METHYL RESIDUAL HOUSE SPRAYING FOR THE CONTROL OF MANSONIA BONNAE, THE VECTOR OF BRugian FILARIASIS. M.S. Chang,\* N. Jute and J. Lah. Vector Borne Diseases Control Programme, Medical Department, Sarawak, MALAYSIA.
- 2:45 33 FIELD TEST OF DNA PROBES FOR BRUGIA MALAYI AND BRUGIA PAHANGI IN INDONESIA. S. Williams, A. Salim, C. Poole, L. McReynolds, Purnomo and F. Partono. Department of Biological Sciences, Smith College, Northampton, MA; New England BioLabs, Beverly, MA; Department of Parasitology, University of Indonesia, JAKARTA.
- 3:00 COFFEE BREAK
- 3:30 34 BLOCKING ACTIVITY LOCALIZES PREDOMINANTLY TO IgG4 ANTIBODIES IN BANCROFTIAN FILARIASIS. R. Hussain,\* R.W. Poindexter and E.A. Ottesen. Aga Khan University, Karachi, PAKISTAN; LPD, National Institutes of Health, Bethesda, MD.
- 3:45 35 IMMUNE RECOGNITION OF RECOMBINANT ONCHOCERCA VOLVULUS ANTIGENS. F.B. Perler, M.W. Southworth,\* I. Matsumura, M. Meda and T.B. Nutman. New England BioLabs, Inc., Beverly, MA; Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.
- 4:00 36 T CELL CLONES AND LINES SPECIFICALLY RECOGNIZING ONCHOCERCA VOLVULUS ANTIGENS. K.F. Colina\* and T.B. Nutman. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.
- 4:15 37 TWO COLOR FLOW CYTOMETRIC ANALYSIS OF LYMPHOCYTE PHENOTYPES IN ONCHOCERCIASIS. D.O. Freedman,\* A. Lujan, C. Gonzales, G. Zea-Flores, E.A. Ottesen and T.B. Nutman. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; the Ministry of Public Health, Guatemala City, GUATEMALA.
- 4:30 38 DOWN REGULATION OF T CELL GROWTH FACTOR PRODUCTION, BUT NOT RECEPTOR EXPRESSION IN BRUGIA PAHANGI INFECTED JIRDS. L.E. Leiva\* and P.J. Lammie. Louisiana State University Medical Center of New Orleans, LA.
- 4:45 39 BRUGIA PAHANGI AND BRUGIA MALAYI: A COMPARISON OF PATHOLOGIC AND IMMUNOLOGIC RESPONSIVENESS IN JIRDS. C.S. McVay,\* T.R. Klej, S.U. Coleman, S.C. Bosshardt and V.A. Dennis. School of Veterinary Medicine, Louisiana State University, Baton Route, LA.

MONDAY AFTERNOON - DECEMBER 5

SCIENTIFIC SESSION C: ANTIGENS OF LEISHMANIA AND TRYPANOSOMES

1:30 PM - 5:15 PM

Congressional

Chairpersons: D.M. Pratt and P. Scott

- 1:30 40 DEVELOPMENTAL MODIFICATION OF LEISHMANIA MAJOR LIPOPHOSPHOGLYCAN DURING METACYCLOGENESIS. D.L. Sacks,\* R. DaSilva and S. Turco. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD; Department of Biochemistry, University of Kentucky Medical Center, Lexington, KY.
- 1:45 41 SEQUENTIAL MODULATION OF PROMASTIGOTE SURFACE MEMBRANE CARBOHYDRATES AND INFECTIVITY OF LEISHMANIA MAJOR. R.L. Jacobson\* and L.F. Schnur. The Kuvim Centre for the Study of Infectious and Tropical Diseases, Hebrew University, Hadassah Medical School, Jerusalem, ISRAEL.
- 2:00 42 T CELL LINES WHICH TRANSFER PROTECTIVE IMMUNITY OR EXACERBATION IN CUTANEOUS LEISHMANIASIS BELONG TO DIFFERENT T HELPER SUBSETS. P. Scott,\* P. Natovitz, E. Pearce and A. Sher. National Institutes of Health, Bethesda, MD.
- 2:15 43 PURIFICATION AND PROPHYLACTIC IMMUNIZATION USING AN 80 KILODALTON PROTEIN FROM LEISHMANIA DONOVANI. A. Clinton White\* and D. McMahon-Pratt. Yale University, New Haven, CT.
- 2:30 44 LEISHMANIA MAJOR: STRUCTURE OF GLYCOSYL-PHOSPHATIDYLINOSITOL ANTIGENS (GPI) RECOGNIZED BY IMMUNE HUMAN SERA. G. Rosen,\* B. Nilsson, M.E. Westerman, D. Sevliver and M.V. Londner. The Kuvim Centre, Hebrew University, Hadassah Medical School, Jerusalem, ISRAEL; Bio Carb, Lund, SWEDEN.
- 2:45 45 COMPARISON OF GLYCOLIPID ANTIGENS OF LEISHMANIA MAJOR AND LEISHMANIA DONOVANI PROMASTIGOTES. D. Sevliver,\* G. Rosen, M.E. Westerman and M.V. Londner. The Kuvim Centre, Hebrew University, Hadassah Medical School, Jerusalem, ISRAEL.
- 3:00 COFFEE BREAK
- 3:30 46 DEVELOPMENT OF MONOCLONAL ANTIBODIES TO LEISHMANIA CHAGASI ANTIGENS WHICH CIRCULATE IN PERSONS WITH VISCERAL LEISHMANIASIS. T.G. Evans,\* M.J. Teixeira, B. Sutherland, Q. Boese and R.D. Pearson. University of Virginia School of Medicine, Charlottesville, VA.
- 3:45 47 CHARACTERIZATION OF ANTIGEN EPITOPES OF INFECTIVE STAGE LEISHMANIA MAJOR PROMASTIGOTES BY USING MONOCLONAL ANTIBODIES IN WESTERN BLOT. S.J. Wu,\* E.D. Rowton, P.V. Perkins, M. Grogil and R.G. Andre. Department of Entomology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.

- 4:00 48 CARBOHYDRATE SPECIFICITY OF ANTI-HEART ANTIBODIES PRODUCED DURING EXPERIMENTAL CHAGAS' DISEASE IN MICE. T.S. McCormick\* and E.C. Rowland. Ohio University College of Osteopathic Medicine, Athens, OH.
- 4:15 49 TRYPANOSOMA CRUZI INVADE MAMMALIAN EPITHELIAL CELLS IN A POLARIZED MANNER. S. Schenkman, V. Nussenzweig and E.S. Robbins. New York University Medical Center, New York, NY.
- 4:30 50 DEVELOPMENTALLY REGULATED TRYPANOSOMA CRUZI TRYPOMASTIGOTE 83 KD GLYCOPROTEIN (GP) BINDS TO MAMMALIAN HOST CELLS IN A LIGAND RECEPTOR INTERACTION MANNER. M.F. Lima\* and F. Villalta. Division of Biomedical Sciences, Meharry Medical College, Nashville, TN.
- 4:45 51 AN ACID pH-DEPENDENT HEMOLYSIN FROM TRYPANOSOMA CRUZI. N.W. Andrews\* and M.B. Whitlow. Department of Pathology, New York University Medical Center, New York, NY.
- 5:00 52 A PROTEIN MARKER FOR ENDOCYTOSIS IN TRYPANOSOMA BRUCEI. S.Z. Shapiro\* and P. Webster. International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, KENYA.

MONDAY AFTERNOON - DECEMBER 5

ACME ANNUAL BUSINESS MEETING

5:00 PM - 5:45 PM

Executive

Chairperson: B.F. Eldridge

5:45 - 6:00 PM

Presentation of the Harry Hoogstraal Medal.

MONDAY AFTERNOON - DECEMBER 5

EFFECTIVE ADVOCACY WORKSHOP: 1989 ASTMH POLITICAL ACTION PLAN

5:30 PM- 7:00 PM

Palladian

Chairperson: S.R. Sagebiel

Time Abst

- 53 K.M. Johnson. Former President, ASTMH.
- 54 S.F. Kuvin. Committee for Public Affairs and Task Force for Political Action, Palm Beach, FL.
- 55 W.E. Small. Executive Director for National Foundation of Infectious Diseases, Bethesda, MD.
- 55a Congressional Staff (to be identified).

MONDAY AFTERNOON - DECEMBER 5

PORTRAITS IN ARBOVIROLOGY

5:00 PM - 7:00 PM

Ambassador

A SPECIAL VIEWING AND DISCUSSION OF THE ASTMH ARCHIVES. Refreshments will be served.

TUESDAY MORNING - DECEMBER 6

POSTER SESSION I WITH CONTINENTAL BREAKFAST

8:00 AM - 10:30 AM

Blue Room

AUTHORS IN ATTENDANCE FROM 8:00 - 10:30 AM

The poster boards will be available in the Blue Room beginning at 7 PM Monday evening. Posters should be set up by 8 AM Tuesday morning and taken down by 12:00 Noon.

ENTOMOLOGY

- 56 VARIATION IN SEASONAL PREVALENCE RATES OF NEUTRALIZING ANTIBODY TO JAMESTOWN CANYON VIRUS IN THREE WHITE-TAILED DEER POPULATIONS AND VECTOR COMPETENCE OF SELECT MOSQUITO SPECIES POTENTIALLY ASSOCIATED WITH THE NATURAL TRANSMISSION CYCLE IN THE UPPER MIDWEST. P.R. Grimstad.\* University of Notre Dame, Notre Dame, IN.
- 57 THE POTENTIAL IMPACT OF MULTIPLE BLOOD-FEEDING BY CULISETA MELANURA ON THE EPIDEMIOLOGY OF EASTERN EQUINE ENCEPHALO- MYELITIS VIRUS. R.A. Anderson,\* J.D. Edman, T.W. Scott and L. Lorenz. University of Massachusetts, Amherst, MA; University of Maryland, College Park, MD.
- 58 MULTIPLE HOST CONTACTS BY CULISETA MELANURA AND TRANSMISSION OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS. T.W. Scott,\* L.H. Lorenz, S.C. Weaver, R.A. Anderson, J.D. Edman and W.S. Romoser. University of Maryland, College Park, MD; University of Massachusetts, Amherst, MA; Ohio University, Athens, OH.
- 59 MAINTENANCE OF AEDEOMYIA SQUAMIPENNIS - A LABORATORY MODEL OF TRANSOVARIAL TRANSMISSION. J.L. Petersen,\* B.E. Outary, and P.H. Peralta. Gorgas Memorial Laboratory, PANAMA.
- 60 EFFECT OF IMMUNE BLOODMEALS ON THE VECTOR COMPETENCE OF AEDES TRISERIATUS FOR LA CROSSE VIRUS. M.S. Godsey, Jr, G.R. DeFoliart, T.M. Yuill. Departments of Entomology and Veterinary Science, and School of Veterinary Medicine, University of Wisconsin, Madison, WI.
- 61 SITES OF RIFT VALLEY FEVER VIRUS INFECTION IN THE PROVENTRICULUS OF ADULT CULEX PIPIENS. K. Lerdthusnee and W.S. Romoser. Ohio University, Athens, OH.

- 62 CONTROL OF GENE EXPRESSION IN THE SALIVARY GLANDS OF VECTOR MOSQUITOES. A.A. James,\* J.V. Racioppi, K. Blackmer and O. Marinotti. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.
- 63 BINDING OF WEE VIRUS TO BRUSH BORDER FRAGMENTS ISOLATED FROM THE MESENTERONAL EPITHELIAL CELLS OF SUSCEPTIBLE AND REFRACTORY CULEX MOSQUITOES. E.J. Houk,\* Y.M. Arcus and J.L. Hardy. School of Public Health, University of California, Berkeley, CA.
- 64 SEASONAL ABUNDANCE OF DENGUE VECTORS IN MANILA, REPUBLIC OF THE PHILIPPINES. G.W. Schultz\*. United States Naval Medical Research Unit No. 2, Manila, PHILIPPINES.
- 65 FAILURE OF ULTRA-LOW VOLUME INSECTICIDE TO PENETRATE TYPICAL RESTING SITES OF AEDES AEGYPTI. P. Reiter,\* M.A. Amador and D.J. Gubler. Dengue Branch, Division of Vector-Borne Viral Diseases, Centers for Disease Control, San Juan, PUERTO RICO.
- 66 INTRADOMICILIARY ACTIVITY OF AEDES AEGYPTI IN SAN JUAN, PUERTO RICO. G.G. Clark,\* H. Seda and D.J. Gubler. Dengue Branch, Division of Vector-Borne Viral Diseases, Centers for Disease Control, San Juan, PUERTO RICO.
- 67 FIELD EVALUATIONS OF A CLOTHING IMPREGNANT AND THREE TOPICAL REPELLENT FORMULATIONS AGAINST TSETSE FLIES IN ZAMBIA. L.L. Sholdt,\* C.E. Schreck, M.I. Mwangelwa, J. Nondo and V.J. Siachinji. USUHS, Bethesda, MD; USDA Insects Affecting Man and Animals Laboratory, Gainesville, FL; Tropical Disease Research Centre, M'Dola, ZAMBIA; Livestock Pest Research Centre, Chilanga, ZAMBIA.
- 68 IDENTIFICATION AND GENETIC CHARACTERIZATION OF CERTAIN VERRUCARUM GROUP SPP. (DIPTERA: PSYCHODIDAE: LUTZOMYIA). R.D. Kreutzer,\* T.M. Palau, A. Morales, C. Ferro, D. Feliciangeli and D.G. Young. Youngstown State University, Youngstown, OH; Instituto Nacional de Salud, Bogota, COLOMBIA; Universidad de Carabobo, Maracay, VENEZUELA; University of Florida, Gainesville, FL.
- 69 POPULATION STRUCTURE OF THE EGYPTIAN CULEX PIPIENS COMPLEX (DIPTERA: CULICIDAE). A.M. Gad\* and A.N. Hassan. Ain Shams University, Cairo, EGYPT.
- 70 INTENSE TRANSMISSION OF THREE MALARIA SPECIES BY ANOPHELES MACULATUS AMONG ORANG ASLI IN EASTERN PERAK, PENINSULAR MALAYSIA. B.A. Harrison,\* D.R. DeLorme, M. Lee and H.M. Savage. United States Army Medical Research Unit, Kuala Lumpur, MALAYSIA; and Institute for Medical Research, Department of Preventive Medicine and Biometrics, USUHS, Bethesda, MD.
- 71 NEW WING CHARACTERS FOR THE IDENTIFICATION OF ANOPHELINE VECTOR GROUPS. R. Wilkerson and E. Peyton. WRBU, Smithsonian, Washington, DC.

## VIROLOGY

- 72 EPIDEMIOLOGY OF HEPATITIS B VIRUS AND SEXUALLY TRANSMITTED DISEASES AMONG PROMISCUOUS HETEROSEXUALS IN SUDAN. M.C. McCarthy,\* J.P. Burans, N.T. Constantine and A. El-Tigani El-Hag. United States Naval Medical Research Unit No. 3, Cairo, EGYPT; Central Public Health Laboratory, Khartoum, SUDAN.
- 73 A SEROSURVEY OF HEPATITIS MARKERS IN THE YEMEN ARAB REPUBLIC. D.A. Scott, J.P. Burans, K.C. Hyams, H.D. Al-Ouzeib, A. Al-Hadad, M. Al-Fadel, Y.R. Nigad, B.K. Arunkumar and J.N. Woody. United States Naval Medical Research Unit No. 3, Cairo, EGYPT; Ministry of Health, Sanaa, YEMEN ARAB REPUBLIC.
- 74 A CROSS-SECTIONAL SEROSURVEY OF VIRAL HEPATITIS MARKERS IN DJIBOUTI. E. Fox,\* E.A. Abbatte, N.T. Constantine, Y. Said-Salah and J.N. Woody. United States Naval Medical Research Unit No. 3, Cairo, EGYPT; International Health Program, University of Maryland School of Medicine, Baltimore, MD; and Ministry of Public Health, DJIBOUTI.
- 75 PREPARATION OF A PURIFIED AND INACTIVATED HEPATITIS A VIRUS VACCINE. K.H. Eckels,\* D.R. Dubois, L.N. Binn, C.T. Rankin, and S.P. O'Neill. Walter Reed Army Institute of Research, Washington, DC; Electro-Nucleonics, Silver Spring, MD.
- 76 INACTIVATED HEPATITIS A VACCINE: FOLLOW-UP AND EVALUATION OF DIFFERENT SCHEDULES. M.H. Sjogren, C.H. Hoke, L.N. Binn, K.H. Eckels, D.H. Dubois, L. Lyde, S. Oaks, R. Marchwicki, R. Lewis, W. Lednar, A. Tsuchida, K. Shafer, R. Chloupek, J. Ticehurst, D.S. Burke and W.H. Bancroft. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; USAMMDA, Frederick, MD; and Ft. Lewis, WA.
- 77 MAPPING OF FUNCTIONAL DOMAINS OF THE DENGUE-2 VIRUS NONSTRUCTURAL GLYCOPROTEIN NS1. J.R. Putnak,\* Department of Virus Diseases, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 78 ANTIGENIC PROPERTIES OF VACCINIA VIRUS RELEVANT TO THE USE OF RECOMBINANT VACCINES. J.W. Hooper,\* S.A. Harrison and A.L. Schmaljohn. United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 79 SURVEY OF HUMANS WITH UNDIFFERENTIATED ACUTE FEBRILE ILLNESS FOR RIFT VALLEY FEVER ANTIBODIES, EGYPT, 1985-1987. A.M. Zaki,\* T.M. Ksiazek, D.M. Watts, M.A. Darwish and C.J. Peters. Research and Training Center for Vector-Borne Disease, Ain Shams University, Cairo, EGYPT; United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; United States Naval Medical Research Unit No. 3, Cairo, EGYPT.
- 80 VIRAL INFECTIONS IN BATS OF GUATEMALA. S.R. Ubico and R.G. McLean\*. Centers for Disease Control, Ft. Collins, CO.
- 81 AEROSOL INFECTION OF RHESUS MONKEYS WITH JUNIN VIRUS. R.H. Kenyon,\* K.T. McKee Jr., P. Zack, C. Crabbs, C. York, A. Frank and C.J. Peters. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

- 82 EVIDENCE FOR THE SPECIFIC BINDING OF RIFT VALLEY FEVER VIRUS TO COMPONENTS OF SOLUBILIZED MOSQUITO TISSUES AND CULTURED CELLS. A.A. Mikhail, M.G. Lozykowski and W.S. Romoser. Ohio University, Athens, OH.
- 83 PROTEINURIA IN MONKEYS INFECTED WITH PROSPECT HILL VIRUS. D.M. Asher,\* A. Collier, A.V. Wolff, R. Yanagihara, L.J. Murphy, K.L. Pomeroy, Z. Eldadah, C.J. Gibbs, Jr. and D.C. Gajdusek. National Institutes of Health, Bethesda, MD.
- 84 INACTIVATED VENEZUELAN EQUINE ENCEPHALOMYELITIS VACCINE: IMMUNOGENICITY OF AND REACTIONS TO A NEW LOT GIVEN AS A BOOSTER. F.J. Malinoski,\* N.A. Popovic, J.A. Mangiafico and G.F. Meadors III. Medical and Disease Assessments Divisions, United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.
- 85 GENETIC REASSORTMENT OF RIFT VALLEY FEVER VIRUS STRAINS IN CELL CULTURE AND IN THE MOSQUITO, CULEX PIPIENS. J.F. Saluzzo, M. Turell, R. Tammariello and J. Smith. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

#### EPIDEMIOLOGY

- 86 THE GEOMETRIC MEAN AND ITS USE FOR EGG COUNT DATA IN SCHISTOSOMIASIS. A.D. Long,\* E.H. Michelson, D.W. Cooper and J.J. Harrington. Harvard School of Public Health, Boston, MA.
- 87 PREVALENCE OF ANTI-DELTA IN HBsAg(+) PATIENTS WITH CIRRHOSIS AND BLOOD DONORS IN CENTRAL TUNISIA. N. Chatti,\* N. Bitar, L. Jemni, S. Said, A. Bchir, A. Jaiden, M. Jemmali, C. Gaudebout and B. Larouze.\* Facultes de Medecine de Sousse et de Monastir, TUNISIA; INSERM U73/IMEA, Hopital Claude Bernard, Paris, FRANCE.
- 88 CAMPYLOBACTER ENTERITIS AMONG AIDS PATIENTS IN LOS ANGELES COUNTY. F.J. Sorvillo, L. Lieb and S.H. Waterman.\* Los Angeles County Department of Health Services, Los Angeles, CA.
- 89 EVALUATION OF MAGAININ ACTIVITY AGAINST PROTOZOAN PATHOGENS. G.L. McLaughlin,\* J.E. Stimac and C.E. Kirkpatrick. University of Illinois, Urbana, IL.
- 90 TUMOR NECROSIS FACTOR IN AEROMONAS-INFECTED RABBITS. N.D. Pacheco,\* D.H. Burr, J. Eckstein and F.M. Rollwagen. Naval Medical Research Institute, Bethesda, MD.
- 91 CONTINUOUS CULTIVATION OF BARTONELLA BACILLIFORMIS. C.R. Latorre,\* I. Columbus, A. Colichon, F.S. Wignall and E.D. Franke. United States Naval Medical Research Institute Detachment, Lima, PERU; Universidad Peruana Cayetano Heredia, Lima, PERU.
- 92 ETIOLOGY OF FEVERS OF UNKNOWN ORIGIN IN PATIENTS ADMITTED TO THE MILITARY HOSPITAL, RAWALPINDI, PAKISTAN. R.E. Krieg,\* J.F. Duncan, J.P. Bryan, B. Awan, A. Ahmed, M. Riaz, S. Nabi, J.E. Sippel and N. Bukhtiar. USUHS, Bethesda, MD; PULSE, AMC, Rawalpindi, PAKISTAN; NMRI, Bethesda, MD; KPL, Gaithersburg, MD.

## CLINICAL TROPICAL MEDICINE

- 93 TUBERCULIN SKIN TEST CONVERSION RATES IN ACTIVE DUTY NAVAL AND MARINE CORPS PERSONNEL, 1980-1986. E.R. Cross,\* R. Wallace and K.C. Hyams. Naval Medical Research Institute, Bethesda, MD; Naval Medical Data Services Center, Bethesda, MD.
- 94 CAMPYLOBACTER INFECTION AMONG INFANTS AND CHILDREN, SANAA, YEMEN ARAB REPUBLIC. R.L. Haberberger, Jr.,\* M. Hussein, M. Ishak and J.N. Woody. United States Naval Medical Research Unit No. 3, Cairo, EGYPT; Ministry of Health, Central Public Health Laboratory and Revolution Hospital, Sanaa, YEMEN ARAB REPUBLIC.
- 95 FIRST CASE REPORT OF INTESTINAL CAPILLARIASIS IN EGYPT. N.S. Mansour,\* F.G. Youssef and E.M. Mikhail. United States Naval Medical Research Unit No. 3, Cairo, EGYPT.
- 96 METRIFONATE OR PRAZIQUANTEL TREATMENT IMPROVES PHYSICAL FITNESS AND APPETITE OF KENYAN SCHOOL BOYS WITH SCHISTOSOMA HAEMATOBIIUM AND HOOKWORM INFECTIONS. L.S. Stephenson,\* M.C. Latham, K.M. Kurz and S.N. Kinoti. Division of Nutritional Sciences, Cornell University, Ithaca, NY; Medical Research Center, a Department of KEMRI, Nairobi, KENYA.
- 97 THE TREATMENT OF ACUTE FASCIOLA HEPATICA INFECTION IN CHILDREN. Z. Farid,\* N. Mansour, M. Kamal, K. Kamal, Y. Safwat and J.N. Woody. United States Naval Medical Research Unit No. 3, and Abbassia Fever Hospital (AFH), Ministry of Health, Cairo, EGYPT.
- 98 HIGH-LEVEL GENTAMICIN RESISTANT ENTEROCOCCI FROM ZIMBABWE. L.S. Mangine,\* M. Barry, J. Gallant and J. Evans Patterson. Medicine and Laboratory Medicine, Yale University School of Medicine, New Haven, CT; University of Zimbabwe, Harare, ZIMBABWE.
- 99 SEROLOGICAL EVALUATION OF PATIENTS WITH EOSINOPHILIA AND HELMINTHIC INFECTIONS: A PRELIMINARY STUDY. N.A. El Masry,\* H. Shaheen, S. Bassily, K. Kamal, Z. Farid and A. Ghaly. United States Naval Medical Research Unit No. 3, Cairo, EGYPT.
- 100 NUTRITIONAL STUDIES ON CONGOLESE CHILDREN. E. Doehring-Schwerdtfeger,\* M. Leichsenring and H.J. Bremer. Kinderklinik der Med. Hochschule, Hannover, FEDERAL REPUBLIC OF GERMANY.
- 101 RESULTS OF 859 SONOGRAPHIC EXAMINATIONS AT A REGIONAL HOSPITAL UNDER TROPICAL CONDITIONS. D. Franke,\* E. Doehring-Schwerdtfeger, G. Mohamed-Ali, I.M. Abdel-Rahim, R. Kardorff, M. Dittrich and J.H.H. Ehrich. Kinderklinik Med. Hochschule, Hannover and Mainz, FEDERAL REPUBLIC OF GERMANY; University of Gezira, Wad Medani, SUDAN.

## GIARDIA

- 102 EFFECT OF DIETARY FIBER ON EXPERIMENTAL GIARDIASIS. G.S. Visvesvara, S.P. Wahlquist and C.T. Harmon. Morehouse School of Medicine and Centers for Disease Control, Atlanta, GA.
- 103 ANALYSIS OF ANTI-GIARDIA ANTIBODIES IN PATIENTS WITH GIARDIASIS. J.P. Nowakowski\* and D.R. Hill. Division of Infectious Diseases, University of Connecticut School of Medicine, Farmington, CT.

- 104 SENSITIVITY OF GIARDIA LAMBLIA TO PROTEIN SYNTHESIS INHIBITORS: CORRELATION WITH SEQUENCE AND STRUCTURE OF RIBOSOMAL RNA. T.D. Edlind.\* Medical College of Pennsylvania, Philadelphia, PA.
- 105 TVF: A SOLUBLE CELL-DETACHING FACTOR SECRETED BY TRICHOMONAS VAGINALIS: W.B. Lushbaugh,\* A.C. Turner and P.C. Klykken. The University of Mississippi Medical Center, Jackson, MS.

#### AMEBIASIS

- 106 CLONING AND CHARACTERIZATION OF THE MAJOR ANTIGENS OF ENTAMOEBA HISTOLYTICA RECOGNIZED BY HUMAN IMMUNE SERA. E.L.W. Kittler,\* W.A. Petri Jr. and J.I. Ravdin. University of Virginia School of Medicine, Charlottesville, VA.

#### CRYPTOSPORIDIUM

- 107 COMMON OCCURRENCE OF ASYMPTOMATIC CRYPTOSPORIDIUM AMONG INFANTS AND TODDLERS IN A NEW YORK CITY DAY CARE CENTER. F.G. Crawford,\* S.H. Vermund, J. Ma and R.J. Deckelbaum. Mt. Sinai Medical Center, Albert Einstein College of Medicine/Montefiore Medical Center; and Columbia University, New York, NY.
- 108 EFFECT OF RECOMBINANT TUMOR NECROSIS FACTOR AND INTERLEUKIN-1 TREATMENT ON THE INTRACELLULAR DEVELOPMENT OF EIMERIA TENELLA. M.H. Kogut and C. Lange. Rutgers University, New Brunswick, NJ.
- 109 THE NUDE MOUSE AS A MODEL FOR CRYPTOSPORIDIOSIS IN THE IMMUNODEFICIENT HOST. M.A. Pentella, B.H. Kwa\* and A.C. Vickery. Lakeland Regional Medical Center, Lakeland, FL; University of South Florida, Tampa, FL.

#### TOXOPLASMOSIS

- 110 DIAGNOSIS OF EXPERIMENTAL MURINE TOXOPLASMOSIS UTILIZING A T. GONDII cDNA PROBE. L.M. Weiss,\* S.A. Udem, H.B. Tanowitz and M. Wittner. Albert Einstein College of Medicine, New York, NY.
- 111 COMPARATIVE EVALUATION OF IFA AND EIA TECHNIQUES IN THE SEROLOGICAL DIAGNOSIS OF TOXOPLASMOSIS: DIFFICULTIES ASSOCIATED WITH SPECIMENS FROM IMMUNOCOMPROMISED PATIENTS. J.A. Kiehlbauch,\* R. Bacina and T.R. Fritsche. University of Washington School of Medicine, Seattle, WA.

#### MISCELLANEOUS

- 112 MYCOBACTERIUM TUBERCULOSIS-SPECIFIC DNA PROBE: SEQUENCE ANALYSIS AND POLYMERASE CHAIN REACTION AMPLIFICATION FOR DIAGNOSIS. R.J. Patel,\* W.M. Meyers, J.C. Samuelson, W.F. Piessens, J.R. David and D.F. Wirth. Department of Tropical Public Health, School of Public Health, Harvard University, Boston, MA; Division of Microbiology, Department of Infectious and Parasitic Disease Pathology, AFIP, Washington, DC.

#### MALARIA - CHEMOTHERAPY

- 113 QUINIDINE AND QUININE AS MODELS OF THE NON-WEAK BASE ACTIVITY OF ANTIMALARIALS. I.Y. Gluzman,\* P.H. Schlesinger and D.J. Krogstad. Washington University, St. Louis, MO.

- 114 REVERSAL OF CHLOROQUINE RESISTANCE IN PLASMODIUM FALCIPARUM FROM VARIOUS GEOGRAPHIC REGIONS. A.M.J. Oduola,\* D.E. Kyle, S.K. Martin and W.K. Milhous. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 115 CALCIUM CHANNEL BLOCKERS AS ADJUNCT TREATMENT OF MALARIA? W.K. Milhous,\* E.F. Boudreau, J. Freeman, L. Pang, D.E. Kyle, A.M.J. Oduola, C.J. Canfield and B.G. Schuster. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 116 IN VITRO ACTIVITY OF DOXYCYCLINE IN COMBINATION WITH QUININE AGAINST BLOOD STAGES OF PLASMODIUM FALCIPARUM. R.G. Taylor,\* W.K. Milhous, D.L. Brown and D.M. Hochstetler. Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD; Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 117 DIETHYLDITHIOCARBAMATE (DDC) ACTS SYNERGISTICALLY WITH COPPER AS AN ANTIMALARIAL. D.G. Heppner,\* S.R. Meshnick, A. Ranz, S. Lu, M. Qian and J.W. Eaton. University of Minnesota, MN; City College of New York, NY; Henan Medical University.
- 118 A SYNERGISTIC DELETERIOUS EFFECT OF ASCORBATE AND COPPER ON THE DEVELOPMENT OF PLASMODIUM FALCIPARUM IN NORMAL AND IN G6PD DEFICIENT ERYTHROCYTES. J. Golenser,\* E. Marva, A. Cohen, P. Saltman and M. Chevion. Departments of Parasitology and Cellular Biochemistry, The Hebrew University, JERUSALEM; Department of Biology, University of California, San Diego, CA.
- 119 EVALUATION OF EXPERIMENTAL COMPOUNDS FOR CAUSAL PROPHYLACTIC ACTIVITY AGAINST MALARIA IN SPOROZOITE INOCULATED MICE. R.G. May and A.L. Ager. Department of Microbiology and Immunology, University of Miami, FL.
- 120 EFFECT OF MEFLOROQUINE AND QINGHAOSU ON THE SPOROGENIC CYCLE OF PLASMODIUM BERGHEI ANKA IN ANOPHELES STEPHENSI MOSQUITOES. R.E. Coleman,\* J.E. Vaughan, D. Hayes, M. Hollingdale, M. Plein and V.E. Do Rosario. Biomedical Research Institute, Department of Entomology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; School of Medicine, University of Maryland, Baltimore, MD.
- 121 TRANSIENT CONTINUATION OF CHLOROQUINE-SENSITIVE PLASMODIUM FALCIPARUM PARASITEMIA IN VOLUNTEERS RECEIVING CHLOROQUINE THERAPY. D.A. Herrington,\* D.F. Clyde, J.R. Murphy, S. Baqar and J.R. Davis. Center for Vaccine Development, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD.
- 122 EFFICACY OF MALARIA PROPHYLAXIS IN PREGNANT WOMEN: THE ROLE OF PATIENT COMPLIANCE VERSUS PARASITE SENSITIVITY. J.W. Wirima,\* D.L. Heymann and R.W. Steketee. Malawi Ministry of Health, Lilongwe, MALAWI; International Health Program Office and Malaria Branch, Centers for Disease Control, Atlanta, GA.

- 123 CHLOROQUINE AND MEFLOROQUINE USED FOR MALARIA PROPHYLAXIS DURING PREGNANCY. R.W. Steketee,\* J. Wirima, D.L. Heymann, C. Khoromana and J.G. Breman. Malaria Branch and International Health Program Office, Centers for Disease Control, Atlanta, GA; Ministry of Health, MALAWI.
- 124 MALARIA CHEMOPROPHYLAXIS STUDY AMONG PEOPLE LIVING IN A MALARIA ENDEMIC AREA OF THE PHILIPPINES. R. Oberst,\* L. Laughlin, N. Sy, A. Alcantara, L. Padre, C. Manaloto, M. Santos and C. Echeverri. United States Naval Medical Research Unit No. 2 and Malaria Control Service, Department of Health, Republic of the Philippines, Manila, PHILIPPINES.
- 125 WHOLE BLOOD LEVELS FOR MEFLOROQUINE AND ACID METABOLITE IN PREGNANT WOMEN IN MALAWI. L. Patchen,\* L. Slutsker, S. Williams, J. Wirima and R. Steketee. Control Technology Branch and Malaria Branch, Centers for Disease Control, Atlanta, GA; Ministry of Health, MALAWI.
- 126 LACK OF EFFICACY OF PYRIMETHAMINE PROPHYLAXIS IN PREGNANT NIGERIAN WOMEN. B.L. Nahlen,\* A. Akintunde, T. Alakija, P. Nguyen-Dinh, O. Ogunbode, L.D. Edungbola, O. Adetoro and J.G. Breman. Malaria Branch, Centers for Disease Control, Atlanta, GA; University of Ilorin, NIGERIA.
- 127 KINETICS OF INTRAMUSCULAR AMOPYROQUINE\* IN HEALTHY SUBJECTS AND MALARIAL PATIENTS. F. Verdier,\* E. Pussard, J. Le Bras, F. Clavier and C. Gaudebout. INSERM U13, Hopital Claude Bernard, Paris, FRANCE.
- 128 A BIOASSAY FOR DETERMINING CYCLOGUANIL CONCENTRATIONS IN HUMAN PLASMA USING PLASMODIUM FALCIPARUM AS THE TEST ORGANISM. C. Lambros,\* V. Navaratnam and G.E. Lewis, Jr. United States Army Medical Research Unit-MALAYSIA; Institute for Medical Research, KUALA LUMPUR; National Drug Research Center, Penang, MALAYSIA.
- 129 CHLOROQUINE AND FANSIDAR RESISTANT FALCIPARUM MALARIA ACQUIRED IN LIBERIA BY AN AMERICAN TRAVELER. P.F. Pierce, A.M.J. Oduola, D.E. Kyle, L. Gerena, L.C. Patchen and W.K. Milhous.\* Georgetown University Medical Center and Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; Centers for Disease Control, Atlanta, GA.
- 130 MALARIA DRUG THERAPY IN ESMERALDAS PROVINCE, ECUADOR. B.L. Nahlen, J.D. Sexton,\* T.K. Ruebush II, I. Caicedo, G. Macias, E.B. Hayes and J.M. Stewart. Divisions of Parasitic and Viral Diseases, Centers for Disease Control, Atlanta, GA; Servicio Nacional de Erradicacion de la Malaria (SNEM), ECUADOR.
- 131 EFFICACY OF A 3-DAY ORAL QUININE TREATMENT FOR P. FALCIPARUM MALARIA IN MADAGASCAR. C. Chougnnet,\* J.P. Lepers, P. DeLoron, F. Verdier, J.A. Ramanamirija, G. Jaureguiberry and P. Coulanges.

TUESDAY MORNING - DECEMBER 6

SYMPOSIUM: THE WATER AND SANITATION DECADE (1981-1990):  
ITS IMPACT ON TROPICAL DISEASE

10:00 AM - 12:00 Noon

Regency

Chairpersons: D.R. Hopkins

<u>Time</u>	<u>Abst</u>	
10:00	132	THE DECADE IN PERSPECTIVE. A. Rotival. UNDP/WHO Coordinator, International Drinking Water Supply and Sanitation Decade, Geneva, SWITZERLAND.
10:30	133	IMPACT ON DIARRHEAL DISEASES: NEW RESULTS AND IMPROVED METHODOLOGY. R. Feacham. Population and Human Resources Department, The World Bank, Washington, DC.
11:00	134	MOVIE: "GUINEA WORM: THE FIERY SERPENT"
11:25	135	SCHISTOSOMIASIS CONTROL AND THE DECADE: ECOLOGICAL APPROACHES TO EVALUATION. F. DeWolfe Miller. School of Public Health, University of Hawaii, Honolulu, HI.
11:55		SUMMARY. D.R. Hopkins.

TUESDAY MORNING - DECEMBER 6

SCIENTIFIC SESSION D: ARBOVIROLOGY - EPIDEMIOLOGY AND PATHOGENESIS

10:00 AM - 12:00 Noon

Palladian

Chairpersons: R. Rico-Hesse and R.E. Shope

<u>Time</u>	<u>Abst</u>	
10:00	136	SEROPREVALENCE OF ANTIBODY (AB) TO ST. LOUIS ENCEPHALITIS (SLE) VIRUS IN AN ADULT MEDICAL AND PEDIATRIC OUTPATIENT POPULATION IN LOS ANGELES COUNTY (LAC). P. Kerndt,* T. Tsai, S. Waterman, K. Iwakoshi, M. Tormey, W. Uyeda, F. Sorvillo, R. Bolin, W. Welch and G. Run. Centers for Disease Control, Atlanta, GA and Fort Collins, CO; Los Angeles County Department of Health Services, Los Angeles, CA.
10:15	137	EPIDEMIOLOGIC ASPECTS OF A WESTERN EQUINE ENCEPHALITIS EPIDEMIC IN THE UNITED STATES, 1987. T.F. Tsai,* W.J. Pape, L.A. Peterson, C. Janney, C.H. Calisher and R.E. Hoffman. Centers for Disease Control, Ft. Collins, CO; Colorado Department of Health, Denver, CO; National Vet. Serv. Laboratory, USDA, Ames, IA; Colorado State University, Department of Environmental Health, Ft. Collins, CO.
10:30	138	HANTAVIRUS INFECTIONS IN HUMANS AND COMMENSAL RODENTS IN SINGAPORE. T.W. Wong,* Y.C. Chan, Y.G. Joo, H.W. Lee, P.W. Lee, R. Yanagihara, C.J. Gibbs and D.C. Gajdusek. National University of Singapore; Korea University, Seoul, KOREA; National Institutes of Health, Bethesda, MD.

- 10:45 139 MARBURG VIRUS: THE SEARCH AT KITUM CAVE. E.D. Johnson,\* J. Morrill, P. Lawyer, P. Tukei, R. Trotter, J. White, B. Hall, M. Kiley, D. Silverstein, R. Zimmerman and B. Johnson. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; US Army Medical Research Unit and Kenya Medical Research Institute, Nairobi, KENYA; Centers for Disease Control, Fort Collins, CO; Nairobi Hospital, NAIROBI; Virus Research Center, KMRI.
- 11:00 140 EPIDEMIOLOGY OF DENGUE VIRUS SEROTYPES 1 AND 2 AS REVEALED BY PRIMER-EXTENSION SEQUENCING. R. Rico-Hesse.\* Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT.
- 11:15 141 ASSOCIATION OF ENCEPHALOPATHY AND HEMORRHAGE WITH BIOPSY PROVEN HEPATIC NECROSIS IN DENGUE TYPE-3 INFECTION IN THAILAND, 1987. B. Innis,\* S. Tanprasertsuk, A. Nisalak, W. Baze, W. Pongritsakda, W. Ovenontach, N. Nimpitakpongse, K. Limboonsuebsai, S. Chhomsai Na Ayuthaya and K. Ung-chusakdi. Armed Forces Research Institute of Medical Sciences, and Ministry of Public Health, Bangkok, THAILAND.
- 11:30 142 EPIDEMIC DENGUE 2 IN THE REPUBLIC OF PALAU. D.J. Gubler,\* S. Deitchman, M. O'Leary, I. Gomez and E. Vergne. Dengue Branch, Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, San Juan, PUERTO RICO.
- 11:45 143 MONOCYTE-INFECTIVITY AS A VIRULENCE MARKER FOR DENGUE-2 VIRUS. S.C. Kliks, C.K. Kent, L.H. Wahl and J.L. Hardy. University of California at Berkeley, Berkeley, CA; National Institutes of Health, Bethesda, MD.

TUESDAY MORNING - DECEMBER 6

SCIENTIFIC SESSION E: FILARIASIS - BIOCHEMISTRY AND MOLECULAR BIOLOGY

10:00 AM - 12:00 Noon

Diplomat

Chairpersons: L.A. McReynolds and P.F. Weller

Time    Abst

- 10:00 144 SYNTHETIC AND NATURALLY OCCURRING RETINOIDS INHIBIT THIRD- TO FOURTH-STAGE LARVAL DEVELOPMENT BY ONCHOCERCA LIENALIS IN VITRO. J.B. Lok,\* R.A. Morris, B.P. Sani, Y.F. Shealy and J.J. Donnelly. University of Pennsylvania, Philadelphia, PA; Southern Research Institute, Birmingham, AL.
- 10:15 145 ISOLATION AND PRELIMINARY CHARACTERIZATION OF GLYCOLIPID AND GLYCOLIPID OLIGOSACCHARIDE RESIDUES OF ADULT ONCHOCERCA GIBSONI. L.H. Semprevivo,\* M.D. Maloney and J.G. Semprevivo. University of Massachusetts, Amherst, MA.

- 10:30 146 IN VIVO AND IN VITRO KILLING OF BRUGIA MALAYI LARVAE AND MICROFILARIAE BY CGP 20 376 (CIBA-GEIGY LIMITED). J.A. Yates, K.L. Hellner\* and G.I. Higashi. University of Michigan, School of Public Health, Department of Epidemiology, Ann Arbor, MI.
- 10:45 147 HYDROGEN PEROXIDE IS TOXIC FOR ONCHOCERCA MICROFILARIAE. H.L. Callahan,\* E.R. James and R.K. Crouch. Medical University of South Carolina, Charleston, SC.
- 11:00 148 THE CHARACTERIZATION OF A HEAT SHOCK PROTEIN 70 (HSP70) GENE OF BRUGIA MALAYI. N.M. Rothstein\* and T.V. Rajan. Departments of Microbiology and Immunology and Pathology, Albert Einstein College of Medicine, Bronx, NY.
- 11:15 149 CHARACTERIZATION OF 2 BRUGIA MALAYI REPEAT FAMILIES. M. Cameron\* and T.V. Rajan. Departments of Pathology and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY.
- 11:30 150 MOLECULAR GENETICS OF FILARIAL MYOSIN. C. Werner,\* N. Rothstein and T.V. Rajan. Departments of Microbiology and Immunology and Pathology, Albert Einstein College of Medicine, Bronx, NY.
- 11:45 151 SEQUENCE AND GENOMIC ORGANIZATION OF THE MAJOR SPERM PROTEIN GENES FROM ONCHOCERCA VOLVULUS. A.L. Scott, J. Dinman,\* D.J. Sussman and S. Ward. Department of Immunology and Infectious Diseases, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD.

TUESDAY MORNING - DECEMBER 6

SCIENTIFIC SESSION F: BIOCHEMISTRY AND MOLECULAR BIOLOGY OF KINETOPLASTIDA

10:00 AM - 12:00 Noon

Empire

Chairpersons: K.P. Chang and S.H. Giannini

Time    Abst

- 10:00 152 PURIFICATION OF GLYCEROL-3-PHOSPHATE OXIDASE FROM THE MITOCHONDRIA OF TRYPANOSOMA BRUCEI. H.S. Bass\* and G.C. Hill. Division of Biomedical Sciences, Meharry Medical College, Nashville, TN.
- 10:15 153 3-METHOXYPHENYLACETIC ACID, AN INHIBITOR OF FUMARATE REDUCTASE IN TRYPANOSOMA BRUCEI, STIMULATES H<sub>2</sub>O<sub>2</sub> PRODUCTION IN INTACT CELLS. J.F. Turrens.\* Department of Biochemistry, College of Medicine, University of South Alabama, Mobile, AL.
- 10:30 154 BIOCHEMISTRY OF PENTOSTAM-RESISTANT LEISHMANIA. J.D. Berman,\* M. King, N. Edwards and M. Grog1. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.

- 10:45 155 METACYCLIC STAGE-SPECIFIC cDNA CLONES OF L. MAJOR AND T. CRUZI. S. Heath,\* D. Sacks, K. Vernick and A. Sher. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD.
- 11:00 156 MOLECULAR KARYOTYPE ANALYSIS OF LEISHMANIA BRAZILIENSIS PANAMENSIS. S.H. Giannini,\* N.G. Saravia and S.S. Curry. Department of Medicine and Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD; Central Internacional de Investigaciones Medicas, Tulane University CIDEIM, Cali, COLOMBIA.
- 11:15 157 INTERFERON-GAMMAS (IFN) INDUCES ALKALINIZATION OF LEISHMANIA CONTAINING PHAGOLYSOMES (PL). D.J. Wyler,\* M.V. Callahan and R.B. Mikkelsen. New England Medical Center Hospitals, and Tufts University School of Medicine, Boston, MA.
- 11:30 158 EXACERBATION OF LEISHMANIA MAJOR INFECTION IN MICE TREATED WITH MONOCLONAL ANTI-INTERFERON GAMMA. M. Belosevic,\* D.S. Finbloom, P.H. Van der Meide and C.A. Nacy. University of Alberta, CANADA; Primate Center TNO, the NETHERLANDS; Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 11:45 159 RESPIRATORY METABOLISM OF METACYCLIC AND EPIMASTIGOTE STAGES OF AFRICAN TRYPANOSOMES. E.J. Biensen\*, R. Kaminsky, P. Webster and W.R. Fish. International Laboratory for Research on Animal Diseases, Nairobi, KENYA.

TUESDAY MORNING - DECEMBER 6

SCIENTIFIC SESSION G: MALARIA - BIOLOGY AND IMMUNOPATHOLOGY

10:00 AM - 12:00 Noon

Executive

Chairpersons: R. Desowitz and S. Langreth

- | <u>Time</u> | <u>Abst</u> |                                                                                                                                                                                                                     |
|-------------|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 10:00       | 160         | FURTHER STUDIES ON EXTRACELLULAR DEVELOPMENT OF <u>PLASMODIUM FALCIPARUM</u> . W. Trager* and J.L. Zung. The Rockefeller University, New York, NY.                                                                  |
| 10:15       | 161         | CHARACTERIZATION OF THE 5.8S RIBOSOMAL RNA CODING DOMAINS FROM <u>PLASMODIUM FALCIPARUM</u> . D. Shippen-Lentz, T. Afroze* and A.C. Vezza. University of Alabama at Birmingham Medical Center, Birmingham, AL.      |
| 10:30       | 162         | ROLE OF THE <u>PLASMODIUM FALCIPARUM</u> MEROZOITE SURFACE ANTIGEN (PF 190-200) IN ERYTHROCYTE INVASION. M.E. Perkins and L.J. Rocco. Laboratory of Biochemical Cytology, The Rockefeller University, New York, NY. |

- 10:45 163 ROLE OF RHOPTRIES IN P. FALCIPARUM MEROZOITE INVASION OF ERYTHROCYTES. T.Y. Sam-Yellowe and M. Murray. Laboratory of Biochemical Cytology, The Rockefeller University, New York, NY.
- 11:00 164 ACTIVATION OF MONOCYTES AND PLATELETS BY MONOCLONAL ANTIBODIES OR PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES BINDING TO THE CD36 SURFACE RECEPTOR IN VITRO. C.F. Ockenhouse,\* C. Magowan and J.D. Chulay. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.
- 11:15 165 PLASMODIUM SPOOROZOITE INTERACTIONS WITH MACROPHAGES IN VITRO: A VIDEOMICROSCOPIC ANALYSIS. J.P. Vanderberg,\* P. Goldie and M.J. Stewart. Department of Medical and Molecular Parasitology, New York University Medical School, New York, NY.
- 11:30 166 MOVEMENT OF A FALCIPARUM MALARIAL PROTEIN THROUGH THE ERYTHROCYTE CYTOPLASM TO THE ERYTHROCYTE MEMBRANE IS ASSOCIATED WITH LYSIS OF THE ERYTHROCYTE AND RELEASE OF GAMETES. I.A. Quakyi,\* Y. Matsumoto, R. Carter, R. Udomsangpetch, M. Aikawa and L.H. Miller. Malaria Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD; Case Western Reserve University, Institute of Pathology, Cleveland, OH; Institute of Pathology, Department of Genetics, University of Edinburgh, SCOTLAND; Department of Immunology, University of Stockholm, SWEDEN.
- 11:45 167 NEW WORLD PRIMATES IN DEVELOPMENT AND EVALUATION OF MALARIA VACCINES. W.E. Collins,\* T.K. Ruebush II and C.C. Campbell. Malaria Branch, Centers for Disease Control, Atlanta, GA.

TUESDAY MORNING - DECEMBER 6

SCIENTIFIC SESSION H: MALARIA - EPIDEMIOLOGY

10:00 AM - 12:00 Noon

Congressional

Chairpersons: C.C. Campbell, S.L. Hoffman and H.-C. Hsieh

Time    Abst

- 10:00 168 ASSESSMENT OF NATURALLY ACQUIRED RESISTANCE TO BLOOD STAGE P. FALCIPARUM IN IRIAN JAYA, INDONESIA. T.R. Jones,\* J.K. Baird, H. Basri, Purnomo and F.P. Paleologo. United States Naval Medical Research Unit No. 2 Detachment, Jakarta, INDONESIA.
- 10:15 169 USE OF DNA PROBES AS EPIDEMIOLOGIC TOOLS IN MALARIA RESEARCH. R.H. Barker, Jr.,\* T. Banchongaksorn and D.F. Wirth. Harvard School of Public Health, Boston, MA; The Malaria Division, Ministry of Public Health, Bangkok, THAILAND.

- 10:30 170 ANTIBODY RESPONSE TO SPOROZOITES IN NATURALLY ACQUIRED PLASMODIUM VIVAX MALARIA IN THAILAND. H.K. Webster, C. Wongsrichanalai, J.B. Gingrich, B. Permpnich, A. Suvarnamani, S. Tulyayon, W.R. Ballou and R.A. Wirtz. United States Army Medical Component, AFRIMS, Bangkok, THAILAND; Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 10:45 171 ONGOING SERO-EPIDEMIOLOGICAL STUDIES ON HUMAN PLASMODIA IN THE STATE OF PARA, AMAZON BASIN, BRASIL. M.E. Arruda,\* A.H. Cochrane, E.H. Nardin and R.S. Nussenzweig. Department of Entomology, Oswaldo Cruz Foundation, Rio de Janeiro, BRASIL; Department of Medicine and Molecular Parasitology, New York University School of Medicine, New York, NY.
- 11:00 172 NATURALLY ACQUIRED ANTIBODIES TO RING-INFECTED ERYTHROCYTE SURFACE ANTIGEN (RESA) AND CIRCUMSPOROZOITE PROTEIN OF PLASMODIUM FALCIPARUM: LACK OF ASSOCIATION WITH PROTECTION AGAINST NEW MALARIA INFECTIONS IN CHILDREN IN WESTERN KENYA. A.D. Brandling-Bennett,\* G.H. Campbell, P. Nguyen-Dinh, P.M. Procell, J.A. Rubatsky, J.S. Odera, C.O. Osanga and J.B.O. Werc. Kenya Medical Research Institute and Division of Vector Borne Diseases, Nairobi, KENYA; Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.
- 11:15 173 ANTIBODIES TO THE PLASMODIUM FALCIPARUM RING-INFECTED ERYTHROCYTE SURFACE ANTIGEN (RESA): SEASONAL PREVALENCE IN TWO KENYAN VILLAGES. P. DeLoron,\* G.H. Campbell, A.D. Brandling-Bennett, J.M. Roberts, I.K. Schwartz, S.J. Odera and C.O. Osanga. Division of Parasitic Diseases, Centers for Disease Control; INSERM U-13, Paris, FRANCE; Kenya Medical Research Institute and Division of Vector-Borne Diseases, KENYA.
- 11:30 174 ANTIBODY TO RESA IN NATIVES AND TRANSMIGRANTS LIVING IN ARSO PIR, IRIAN JAYA. J.K. Baird,\* T.R. Jones, B. Leksana and B.A. Annis. United States Naval Medical Research Unit #2, Jakarta Detachment, INDONESIA.
- 11:45 175 DETECTION OF ANTIBODIES IN HUMANS OF DIFFERENT MALARIA-ENDEMIC REGIONS TO SYNTHETIC PEPTIDES DERIVED FROM PLASMODIUM FALCIPARUM EXOANTIGENS. S. Montenegro-James,\* M.A. James, S.J. Ma, I. Kakoma, O. Noya, F. Riggione and M. Ristic. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL; Universidad Central de Venezuela, Instituto de Medicina Tropical, Caracas, VENEZUELA.

TUESDAY AFTERNOON - DECEMBER 6

SCIENTIFIC SESSION I: SCHISTOSOMIASIS - GENERAL

1:15 PM - 3:15 PM

Regency

Chairpersons: P. Basch and W. Michael Kemp

- | <u>Time</u> | <u>Abst</u> |                                                                                                                                                                                                                                                                                                                                                                                                      |
|-------------|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1:15        | 176         | LIPID STIMULATION OF <u>SCHISTOSOMA MANSONI</u> CERCARIAE IS MEDIATED BY ARGENTOPHILIC PAPILLAE ON CERCARIAL SURFACES. C.L. King* and G.I. Higashi. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; The Department of Epidemiology, University of Michigan, Ann Arbor, MI.                                                                                            |
| 1:30        | 177         | THE EFFICACY OF TOPICALLY APPLIED NICLOSAMIDE AT PREVENTING SCHISTOSOMIASIS MANSONI IN RHESUS MONKEYS. R.E. Miller,* L. Lightner, C.B. Clifford, W.A. Reid, Jr., D. Jones and K.R. Witter. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.                                                                                                                  |
| 1:45        | 178         | RISING SALINE CONCENTRATION SIGNALS CERCARIAE OF <u>S. MANSONI</u> TO TRANSFORM TO SCHISTOSOMULA. J.C. Samuelson* and L. Stein. Departments of Tropical Public Health, Harvard School of Public Health and Pathology, Brigham and Women's Hospital, and Comm. on Cell and Developmental Biology, Harvard Medical School, Boston, MA.                                                                 |
| 2:00        | 179         | EXPRESSION AND ROLE OF CYSTEINE PROTEINASES IN <u>IN VITRO</u> CULTURED <u>SCHISTOSOMA MANSONI</u> SCHISTOSOMULA. C.L. Chappell, M.H. Dresden and K.S. Zerda. Department of Biochemistry, Baylor College of Medicine, Houston, TX.                                                                                                                                                                   |
| 2:15        | 180         | ISOLATION AND CHARACTERIZATION OF A CYSTEINE PROTEINASE FROM <u>FASCIOLA HEPATICA</u> ADULT WORMS. A.A. Rege,* P.R. Herrera, M. Lopez and M.H. Dresden. Department of Biochemistry, Baylor College of Medicine, Houston, TX; Department of Biochemistry and Institute of Tropical Medicine, Universidad Peruana Cayetano Heredia, Department of Biochemistry, Universidad de San Marcos, Lima, PERU. |
| 2:30        | 181         | PROTEIN PHOSPHORYLATION DURING TRANSFORMATION OF CERCARIAE TO SCHISTOSOMULA OF <u>SCHISTOSOMA MANSONI</u> . P.M. Wiest,* W.D. Bowen and G.R. Olds. Brown University and the Miriam Hospital, Providence, RI.                                                                                                                                                                                         |
| 2:45        | 182         | ULTRASONOGRAPHIC EVALUATION OF THE MORBIDITY INDUCED BY <u>SCHISTOSOMA MANSONI</u> INFECTION IN CHILDHOOD. R. Kardorff,* E. Doehring-Schwerdtfeger, K.M. Abdel-Rahim, G. Mohamed-Ali, C. Kaiser, M. El Sheikh and J.H.H. Ehrlich. Kinderklinik Med. Hochschule, Hannover, FEDERAL REPUBLIC OF GERMANY; Wad Medani, SUDAN.                                                                            |

3:00 183 ULTRASONOGRAPHY OF SCHOOL CHILDREN WITH SCHISTOSOMIASIS  
MANSONI. M.F. Abdel-Wahab,\* G. Esmat, S.I. Narooz, A. Yosery,  
J.P. Struewing, and G.T. Strickland. Cairo University Faculty  
of Medicine, Cairo, EGYPT; University of Maryland School of  
Medicine, Baltimore, MD.

TUESDAY AFTERNOON - DECEMBER 6

SCIENTIFIC SESSION J: WORKSHOP: LATE BREAKING ADVANCES IN MOLECULAR BIOLOGY

1:15 PM - 3:00 PM

Palladian

Chairpersons: P. LoVerde and T.F. McCutchan

Persons interested in presenting at this session should contact Dr.  
LoVerde (716-831-2459) or Dr. McCutchan (301-496-6149) within 3 weeks of the  
meeting or before noon on Monday December 5th.

Abst

184-190

TUESDAY AFTERNOON - DECEMBER 6

SCIENTIFIC SESSION K: ARBOVIROLOGY - DIAGNOSIS AND TAXONOMY

1:30 PM - 3:00 PM

Diplomat

Chairpersons: C.H. Calisher and T.G. Ksiazek

Time Abst

- 1:30 191 EVALUATION OF FILTER HYBRIDIZATION FOR THE DETECTION OF RIFT  
VALLEY FEVER VIRUS RNA IN HUMAN SERUM FROM THE RECENT  
MAURITANIA EPIDEMIC. F.K. Knauert,\* J.M. Meegan, A. Jouan, B.  
Le Guenzo, T.G. Ksiazek, C.J. Peters and J.P. Digoutte.  
Disease Assessment Division, United States Army Medical  
Research Institute of Infectious Diseases, Ft. Detrick, MD;  
Pasteur Institute, Dakar, SENEGAL.
- 1:45 192 PRELIMINARY EVIDENCE THAT PHLEBOTOMUS FEVER AND UUKUNIEMI  
SEROGROUP VIRUSES ARE ANTIGENICALLY RELATED: PROPOSED  
INCLUSION IN AN EXPANDED GENUS PHLEBOVIRUS. C.H. Calisher,\*  
D.H.L. Bishop and R.E. Pettersson. Centers for Disease  
Control, Fort Collins, CO; NERC Institute of Virology, Oxford,  
UNITED KINGDOM; Ludwig Institute of Cancer Research,  
Karolinska Institute, Stockholm, SWEDEN.
- 2:00 193 NUCLEIC ACID BLOT HYBRIDIZATION TECHNIQUES FOR DETECTION AND  
SURVEILLANCE OF BLUETONGUE VIRUS. R.J. Schoepp,\* J.F. Bray,  
F.R. Holbrook, C.D. Blair, P. Roy and B.J. Beaty. Department  
of Microbiology, Colorado State University, Fort Collins, CO;  
USDA-ARS, Laramie, WY; Department of Environmental Health,  
University of Alabama at Birmingham, AL.

- 2:15 194 DETECTION OF DENGUE VIRUS IN MOSQUITOES AND HUMAN SERUM BY NUCLEIC ACID HYBRIDIZATION. K. Olson,\* C. Blair, R. Padmanabhan and B. Beaty. Department of Microbiology, Colorado State University, Fort Collins, CO; Department of Biochemistry, University of Kansas Medical Center, Kansas City, KS.
- 2:30 195 DEVELOPMENT OF A PLAQUE-IMMUNOBLOTTING PROCEDURE FOR FLAVIVIRUSES. P.L. Summers,\* D.R. Dubois, W. Houston Cohen, R. McN. Scott and K.H. Eckels. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 2:45 196 DENGUE HEMORRHAGIC FEVER IN JAKARTA, INDONESIA. C.R. Bartz,\* C. Maroef, K.S. Tatang, W. Hansa, R. Tan and A. Sie. United States Naval Medical Research Unit No. 2 Detachment and Sumber Waras Hospital, Jakarta, INDONESIA.
- 3:00 197 RIFT VALLEY FEVER AMONG DOMESTIC ANIMALS IN THE RECENT WEST AFRICAN OUTBREAK. T.G. Ksiazek,\* A. Jouan, J.M. Meegan, B. LeGuanno, M.L. Wilson, C.J. Peters, J.P. Digoutte, M. Guillaud, N.O. Merzoug and O.I. Touray. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; Institute Pasteur, Dakar, SENEGAL; IEMVT, Cedex, FRANCE; CNERV, Islamic Republic of Mauritania, Department of Animal Health and Production, Abuko, THE GAMBIA.

TUESDAY AFTERNOON - DECEMBER 6

SCIENTIFIC SESSION L: MALARIA - EXOERYTHROCYTIC ANTIGENS

1:30 PM - 3:00 PM

Empire

Chairpersons: J. Murphy and I. Quakyi

Time    Abst

- 1:30 198 PLASMODIUM FALCIPARUM SPOROZOITES RECOGNIZE A 55-60K HUMAN HEPATOCYTE PUTATIVE RECEPTOR. J. vanPelt, S.B. Aley, M.R. Hollingdale, J.P. Verhave and S.H. Yap. University of Nijmegen Medical School, THE NETHERLANDS; Biomedical Research Institute, Rockville, MD.
- 1:45 199 MONOCLONAL ANTIBODIES (MABs) REACTING WITH CIRCUMSPOROZOITE (CS) PROTEINS OF THE PLASMODIUM CYNOMOLGI COMPLEX AND OF P. KNOWLESI INHIBIT THE IN VITRO DEVELOPMENT OF EXOERYTHROCYTIC STAGES OF P. CYNOMOLGI BASTIANELLII. P. Millet,\* K.K. Kamboj, A.H. Cochrane, W.E. Collins and P. Nguyen-Dinh. Malaria Branch, Centers for Disease Control, Atlanta, GA; Department of Medical and Molecular Parasitology, New York University, New York, NY.
- 2:00 200 INHIBITION OF IN VITRO DEVELOPMENT OF PLASMODIUM BERGHEI LIVER STAGE PARASITES BY SPLEEN CELLS FROM MICE IMMUNIZED WITH IRRADIATION ATTENUATED SPOROZOITES. D. Isenberger,\* G. Long, W.R. Ballou and S.L. Hoffman. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; Naval Medical Research Institute, Bethesda, MD.

- 2:15 201 PLASMODIUM BERGHEI: EXPRESSION OF CIRCUMSPOROZOITE PROTEIN DURING EXOERYTHROCYTIC DEVELOPMENT IN HEPG2-A16 HEPATOMA CELLS. C.T. Atkinson,\* M. Aikawa, S.B. Aley and M.R. Hollingdale. Case Western Reserve University, Cleveland, OH; Biomedical Research Institute, Bethesda, MD.
- 2:30 202 PEPTIDE DERIVED FROM THE PLASMODIUM FALCIPARUM LIVER STAGE SPECIFIC ANTIGEN ELICITS ANTIBODIES THAT REACT WITH P. FALCIPARUM SPOROZOITES AND P. BERGHEI SPOROZOITES AND EXOERYTHROCYTIC STAGES. M.R. Hollingdale,\* S.B. Aley, A. Appiah, M. Aikawa and C. Atkinson. Biomedical Research Institute, Rockville, MD; Case Western University, Cleveland, OH.
- 2:45 203 IMMUNIZATION WITH IRRADIATED PLASMODIUM BERGHEI SPOROZOITES DOES NOT PROTECT AGAINST CHALLENGE WITH LIVER STAGE MEROZOITES: A REDEFINITION OF STAGE SPECIFICITY OF SPOROZOITE VACCINES. G. Long, L. Loomis, W.R. Ballou and S.L. Hoffman. Naval Medical Research Institute, Bethesda, MD; Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.

TUESDAY AFTERNOON, DECEMBER 6

SCIENTIFIC SESSION M: TROPICAL VETERINARY MEDICINE

1:15 PM - 3:15 PM

Executive

Chairpersons: P. Alm and J.C. Williams

Time    Abst

- 1:15 204 COMPARING THE RELATIVE POTENTIAL OF RODENTS AS RESERVOIRS OF THE LYME DISEASE SPIROCHETE (BORRELIA BURGENDORFERI). T.N. Mather,\* M.L. Wilson, S.I. Moore, J.M.C. Ribeiro and A. Spielman. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.
- 1:30 205 ISOLATION OF A MAJOR ANAPLASMA MARGINALE PROTEIN FOR USE IN A DOT-ELISA FOR THE SERODIAGNOSIS OF ANAPLASMOSIS. S. Montenegro-James,\* S.J. Ma and M. Ristic. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL.
- 1:45 206 AN EPIZOOTIC OF URBAN CANINE RABIES IN MEXICO. T.R. Eng,\* H.E. Talamante, D.B. Fishbein, G.M. Baer, D.B. Hall, G.F. Chavez, J.G. Dobbins, J. Carrasco and F.J. Muro. Division of Viral Diseases, Division of Birth Defects and Developmental Disabilities, Centers for Disease Control, Atlanta, GA; Servicios Medicos de Sonora, MEXICO; El Paso Field Office, Pan American Health Organization.
- 2:00 207 A UNIQUE SPOTTED FEVER GROUP RICKETTSIA FROM HUMANS IN JAPAN. T. Uchida,\* X.J. Yu, T. Uchiyama and D.H. Walker. University of Tokushima School of Medicine, Tokushima, JAPAN; The University of Texas Medical Branch, Galveston, TX.

- 2:15 208 ADAPTATION OF EHRLICHIA CANIS TO GROWTH IN PRIMARY HUMAN MONOCYTE CELL CULTURES. C.J. Holland,\* I. Kakoma and M. Ristic. University of Illinois College of Veterinary Medicine, Urbana, IL.
- 2:30 209 HUMAN RICKETTSIA TYPHI INFECTION IN THE SINAI, EGYPT. R. Faris,\* M. Kenawi, G.A. Sattar, A.J. Saah and A.F. Azad. Ain Shams University, Cairo, EGYPT; Johns Hopkins School of Hygiene and Public Health; University of Maryland, Baltimore, MD.
- 2:45 210 THE DEVELOPMENT OF ANAPLASMA MARGINALE IN SALIVARY GLANDS OF DERMACENTOR ANDERSONI. K.M. Kocan,\* W. Goff, D. Stiller, A.F. Barbet, W. Edwards, S.A. Ewing, J.A. Hair, S.J. Barron and T.C. McGuire. Oklahoma State University, Stillwater, OK; USDA-ARS Animal Disease Research Unit and Washington State University, Pullman, WA; University of Idaho, Moscow, ID; University of Florida, Gainesville, FL.
- 3:00 211 MAINTENANCE OF A PARALLEL TRANSMISSION CYCLE FOR BORRELIA BURGDORFERI IN RABBITS BY IXODES DENTATUS. S.R. Telford III\* and A. Spielman. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

TUESDAY AFTERNOON - DECEMBER 6

SCIENTIFIC SESSION N: PARASITIC ENTOMOLOGY

1:30 PM - 3:00 PM

Congressional

Chairperson: R.F. Beach

Time    Abst

- 1:30 212 THE EFFECT OF SPOROZOITE DENSITY ON MALARIA TRANSMISSION BY PLASMODIUM FALCIPARUM- AND P. VIVAX-INFECTED ANOPHELES ALBIMANUS IN GUATEMALA. R.F. Beach,\* C. Cordon-Rosales and E. Molina. Medical Entomology Research and Training Unit/GUATEMALA; Division of Parasitic Diseases; Centers for Disease Control, Atlanta, GA.
- 1:45 213 QUANTITATION OF MALARIA SPOROZOITES TRANSMITTED IN VITRO DURING SALIVATION BY WILD NATURALLY INFECTED AFROTROPICAL ANOPHELES. J.C. Beier,\* F.K. Onyango, J.K. Koros, M. Ramadhan, R. Ogwang, C.M. Asiago, C.K. Koech and C.R. Roberts. Kenya Medical Research Institute and United States Army Medical Research Unit, Nairobi, KENYA.
- 2:00 214 A DIPSTICK, DOT-ELISA, ASSAY FOR THE RAPID, FIELD IDENTIFICATION OF MOSQUITO BLOODMEAL SOURCES. H.M. Savage,\* J.F. Duncan, D.R. Roberts and L.L. Sholdt. Department of Preventive Medicine and Biometrics, USUHS, Bethesda, MD.

- 2:15 215 LABORATORY AND FIELD MICROPLATE ASSAY DETECTION OF DDT RESISTANCE IN ANOPHELINES. W.G. Brogdon,\* R.F. Beach, J. Alarcon and A.M. Barber. Malaria Branch and Medical Entomology Research and Training Unit/GUATEMALA; Centers for Disease Control, Atlanta, GA; Servicio Nacional de Erradicacion de La Malaria, Guayaquil, ECUADOR.
- 2:30 216 MONITORING ANOPHELES FREEBORNI LARVAL POPULATION DENSITIES IN CALIFORNIA RICE FIELDS USING REMOTE SENSING TECHNOLOGY. M.J. Pitcairn,\* E. Rejmankova, B. Wood and R.K. Washino. University of California, Davis, CA; NASA/Ames Research Center, Moffett Field, CA.
- 2:45 217 HEMOLYMPH PROTEIN ALTERATIONS IN MOSQUITOES DURING AN IMMUNE RESPONSE TO FILARIAL WORMS. B.T. Beerntsen\* and B.M. Christensen. University of Wisconsin, Madison, WI.

TUESDAY AFTERNOON - DECEMBER 6

ASTMH PRESIDENTIAL ADDRESS

3:30 PM - 4:30 PM

Regency

- 218 A PATHOLOGIST'S LOOK AT INFECTIOUS DISEASE. J.K. Frenkel. Department of Pathology, Kansas University Medical Center, Kansas City, KS.

TUESDAY AFTERNOON - DECEMBER 6

ANNUAL ASTMH BUSINESS MEETING

4:30 PM

Regency

Chairpersons: J.K. Frenkel and W.A. Sodeman, Jr.

WEDNESDAY MORNING - DECEMBER 7

POSTER SESSION II WITH CONTINENTAL BREAKFAST

8:00 AM - 10:30 AM

Blue Room

AUTHORS IN ATTENDANCE FROM 8:00 - 10:30 AM

The poster boards will be available in the Blue Room beginning at 7 PM Tuesday evening. Posters should be set up by 8 AM Wednesday morning and taken down by 12:00 Noon.

MALARIA - BIOLOGY AND PATHOLOGY

- 219 ATTEMPTED IN VITRO CULTIVATION OF PLASMODIUM VIVAX USING HUMAN RETICULOCYTES. A.A. Adelugba,\* J.R. Murphy, V. Pawar, S. Baqar and J. Davis. Program in Medical Technology and Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD.

- 220 Ca<sup>2+</sup>- AND cAMP-DEPENDENT PROTEIN KINASES IN PLASMODIUM FALCIPARUM. L.K. Read\* and R.B. Mikkelsen. Tufts University, Boston, MA; Department of Radiation Oncology, Tufts-New England Medical Center, Boston, MA.
- 221 ROLE OF CYTOPLASMIC VESICLES IN P. FALCIPARUM INFECTED ERYTHROCYTES. Z. Etzion and M.E. Perkins. Rockefeller University, New York, NY.
- 222 HEREDITARY SPHEROCYTOSIS ASSOCIATED WITH SPECTRIN DEFICIENCY DOES NOT SUSTAIN THE GROWTH OF PLASMODIUM FALCIPARUM. S. Schulman,\* E.F. Roth, Jr., R. Schwartz, A. Rybicki, B. Cheng and R.L. Nagel. Department of Natural Sciences, Baruch College, CUNY, New York, NY; Division of Hematology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY.
- 223 PLASMODIUM FALCIPARUM: OOKINETE PENETRATION AND OOCYST DEVELOPMENT IN ANOPHELINE MOSQUITOES. J.F.G.M. Meis, G. Pool, G.J.V. Gemert, A.H.W. Lensen and T. Ponnudurai. Department of Medical Parasitology, University of Nijmegen, The NETHERLANDS.
- 224 P. FALCIPARUM ASSOCIATED PLACENTAL PATHOLOGY - A LIGHT AND ELECTRON-MICROSCOPICAL AND IMMUNOHISTOLOGICAL STUDY. M. Yamada,\* R. Steketee, M. Kida, C. Abramowsky, J. Wirima, D. Heymann, J. Breman and M. Aikawa. Case Western Reserve University, Cleveland, OH; Centers for Disease Control, Atlanta, GA; Ministry of Health, MALAWI.
- 225 CHARACTERIZATION OF A MODEL OF PREGNANCY-ASSOCIATED MALARIAL PATHOPHYSIOLOGY: PLASMODIUM BERGHEI IN THE WHITE RAT. R.S. Desowitz,\* K. Shida, L. Pang and G. Buchbinder. University of Hawaii and Tripler Army Medical Center, Honolulu, HI.

#### MALARIA - ERYTHROCYTIC ANTIGENS

- 226 COMPARISON OF THE BIOLOGIC ACTIVITY OF ANTIBODIES SPECIFIC TO A PLASMODIUM FALCIPARUM 83 KD EXOANTIGEN AND A SYNTHETIC PEPTIDE DERIVATIVE DRUG USING AN IN VITRO NEUTRALIZATION ASSAY. C.J. Fajfar-Whetstone,\* M.A. James and M. Ristic. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL.
- 227 A STRUCTURE-FUNCTION MODEL FOR PLASMODIUM FALCIPARUM 175-KD ERYTHROCYTE BINDING ANTIGEN. J.D. Haynes,\* P.A. Orlandi, M. Zegans, F.W. Klotz and J.D. Chulay. Department of Immunology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 228 IDENTIFICATION OF A RHOPTYR-ASSOCIATED ANTIGEN OF THE SIMIAN MALARIA PARASITE PLASMODIUM FRAGILE WITH MONOCLONAL ANTIBODIES. P.M. Procell,\* P. DeLoron, W.E. Collins and P. Nguyen-Dinh. Malaria Branch, Centers for Disease Control, Atlanta, GA.
- 229 IMMUNOGENICITY OF NATIVE P. FALCIPARUM GP195 IN FCA VERSUS THE COMBINATION OF B30-MDP, LA-15-PH, AND TOM IMMUNOMODULATORS. L.Q. Tam,\* G.S.N. Hui, S. Kotani, T. Shiba, S. Kusumoto and W.A. Siddiqui. Department of Tropical Medicine, University of Hawaii, Honolulu, HI; Osaka College of Medical Technology, Osaka, JAPAN.

- 230 ALBUMIN BINDING COMPONENTS OF PLASMODIUM BERGHEI: NATURE OF THE COMPONENTS, THEIR INTERACTION WITH ALBUMIN AND WITH ANTIPLASMODIAL ANTIBODY. G. Green IV\* and J.P. Kreier. The Ohio State University, Columbus, OH.
- 231 INFECTION WITH PLASMODIUM MALARIAE ELICITS ANTIBODIES WHICH REACT WITH PLASMODIUM BRASILIANUM-INFECTED ERYTHROCYTE MEMBRANES. A.J. Sulzer,\* R.A. Cantella and P. Millet. Malaria Branch, Centers for Disease Control; Atlanta, GA; Universidad Peruana Cayetano Heredia, Lima, PERU.
- 232 CHARACTERIZATION OF A 50 KD ANTIGEN FOUND IN IMMUNE CLUSTERS OF PLASMODIUM FALCIPARUM MEROZOITES. A.W. Thomas,\* D.A. Carr, J.A. Lyon and J.D. Chulay. Department of Immunology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 233 IMMUNOGENICITY OF A CARRIER-FREE SYNTHETIC PEPTIDE COMPLEX DERIVED FROM A 70 KD PLASMODIUM FALCIPARUM EXOANTIGEN. M.A. James,\* S. Montenegro-James,\* C.J. Fajfar-Whetstone, S.J. Ma and M. Ristic. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL.

#### MALARIA - SPOROZOITES

- 234 THE APPEARANCE AND FATE OF THREE DIFFERENT SPOROZOITE ANTIGENS OF PLASMODIUM YOELII TRACKED BY IMMUNOELECTRON MICROSCOPY. R.L. Beaudoin,\* C. Atkinson, L.A. Beaudoin, M. Sedegah, Y. Charoenvit and M. Aikawa. NMRI, Bethesda, MD; Case Western Reserve University, Cleveland, OH; PAHO, Washington, DC.
- 235 PRESENTATION OF A SYNTHETIC PLASMODIUM BERGHEI CIRCUMSPOROZOITE PEPTIDE CONJUGATE ON THE SURFACE OF GM1-BEARING LIPOSOMES. L.D. Loomis, R.L. Richards, J.C. Sadoff, C.R. Alving, W.T. Hockmeyer and W.R. Ballou. Departments of Immunology and Membrane Biochemistry, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 236 RESTRICTION OF MURINE T CELL REPERTOIRES TO PLASMODIUM BERGHEI CIRCUMSPOROZOITE PROTEIN AS A FUNCTION OF ANTIGEN PROCESSING. U. Krzych,\* H. Link, T. Jareed and W.R. Ballou. Department of Biology, Catholic University of America and Department of Immunology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 237 PREVALENCE OF CIRCUMSPOROZOITE ANTIBODIES TO PLASMODIUM VIVAX IN PENINSULAR MALAYSIA: HUMAN IMMUNE SERA REACT WITH NST81V20 A RECOMBINANT VIVAX SPOROZOITE VACCINE CANDIDATE. M. Lee,\* D.R. Davis, W.R. Ballou, G.F. Wasserman and G.E. Lewis, Jr. United States Army Medical Research Unit-MALAYSIA; Institute for Medical Research, KUALA LUMPUR; Department of Immunology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; Department of Protein Biol, Smith, Kline, French Laboratories, SWEDENLAND.
- 238 A MODEL FOR DETAILED STUDIES ON THE SPOROGENY OF MALARIA. V.E. do Rosario,\* R. Coleman, P. Leland, M. Hollingdale, A. Appiah and J. Vaughan.\* Biomedical Research Institute, Rockville, MD; Department of Entomology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; University of Maryland, Baltimore, MD.

- 239 ULTRASTRUCTURAL LOCALIZATION OF CIRCUMSPOROZOITE PROTEIN IN P. CYNOMOLGI EXOERYTHROCYTIC SCHIZONTS. C.T. Atkinson,\* P. Millet, A. Cochrane, W.E. Collins and M. Aikawa. Institute of Pathology, Case Western Reserve University, Cleveland, OH; Malaria Branch, Centers for Disease Control, Atlanta, GA; New York University, New York, NY.
- 240 ADOPTIVE TRANSFER OF PROTECTIVE IMMUNITY TO PLASMODIUM YOELII SPOOROZOITES. S.J. Pancake,\* Y. Charoenvit, M. Sedegah, R.L. Beaudoin and S.L. Hoffman. Infectious Disease Department, Naval Medical Research Institute, Bethesda, MD.
- 241 CIRCUMSPOROZOITE PROTEINS OF MALARIA SPOOROZOITES APPEAR TO BE SECRETORY PROTEINS. M.J. Stewart,\* A.H. Cochrane and J.P. Vanderberg. Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY.

MALARIA - CLINICAL AND EPIDEMIOLOGICAL ASPECTS

- 242 PROGRESS IN THE CONTROL OF MALARIA IN ZIMBABWE. P. Taylor.\* Blair Research Laboratory, Causeway, ZIMBABWE.
- 243 SPECIFIC, NON-ISOTOPIC DETECTION OF PLASMODIUM FALCIPARUM IN BLOOD SMEARS USING ENZYME-LINKED SYNTHETIC DNA. J.E. Marich,\* J.L. Ruth, C.J.T.F. Whetstone, W.E. Collins and G.L. McLaughlin. Molecular Biosystems Inc., San Diego, CA; University of Illinois, Urbana, IL; Centers for Disease Control, Atlanta, GA.
- 244 PROGNOSTIC INDICATORS IN PEDIATRIC CEREBRAL MALARIA. T.E. Taylor\* and M.E. Molyneux. Michigan State University College of Osteopathic Medicine, East Lansing, MI; The Liverpool School of Tropical Medicine, Liverpool, UKRAINE.
- 245 RAPID DIAGNOSIS OF MALARIA BY ACRIDINE ORANGE STAINING OF CENTRIFUGED PARASITES. L.S. Rickman, G. Long, R.B. Oberst, J.E. Egan, T.M. Cosgriff, J.D. Chulay and S.L. Hoffman. Naval Medical Research Institute, Bethesda, MD; United States Naval Medical Research Unit No. 2, Manila, PHILIPPINES; Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; United States Army Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 246 PREDICTION OF MALARIA TRANSMISSION POTENTIAL IN CHIAPAS, MEXICO THROUGH USE OF NASA REMOTE-SENSING TECHNOLOGY. D. Strickman,\* D. Roberts, H. Savage, E. Rejmankova, R. Castro, J. Jimenez, R. Wilkerson, M. Rodriguez and L. Legters. WRBU, Smithsonian; USUHS, Bethesda, MD; University of California at Davis, CA; CIP, Tapachula, MEXICO.
- 247 ADAPTATION AND OPTIMIZATION OF THE FALCON ASSAY SCREENING TEST (FAST-ELISA) FOR TWO-SITE, SINGLE ANTIBODY DETECTION OF PLASMODIUM VIVAX SPOOROZOITES IN INFECTED MOSQUITOES. G.H. Campbell,\* J.D. Sexton, P.M. Procell and W.E. Collins. Malaria Branch, Centers for Disease Control, Atlanta, GA.

- 248 SEROEPIDEMIOLOGY OF ANTIBODIES TO THE MAJOR MEROZOITE SURFACE PRECURSOR PROTEIN OF PLASMODIUM FALCIPARUM (GP195) FROM AN ENDEMIC AREA OF THE PHILIPPINES. K. Kramer,\* D. Moffitt, W. Siddiqui and R. Oberst. Department of Tropical Medicine, University of Hawaii, Honolulu, HI; United States Naval Medical Research Unit No. 2, Manila, PHILIPPINES.
- 249 GAMETOCYTOCIDAL EFFECT OF THE ANTI-MALARIAL DRUG QINGHAOSU ON PLASMODIUM FALCIPARUM. N. Kumar\* and H. Zheng. Immunology and Infectious Diseases, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD.

#### FILARIASIS

- 250 HTLV-1 TRANSFORMED T CELL CLONES FROM A PATIENT WITH TROPICAL PULMONARY EOSINOPHILIA: IDENTIFICATION OF A TH2 HUMAN EQUIVALENT. T.B. Nutman,\* C. Steel and G. Delespesse. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; University of Montreal, CANADA.
- 251 DETECTION OF A PHOSPHORYLCHOLINE-CONTAINING PARASITE ANTIGEN IN SERA FROM BRUGIA MALAYI-INFECTED JIRDS. G.J. Weil.\* Washington University School of Medicine, St. Louis, MO.
- 252 IMMUNOREACTIVITY OF CLONED BRUGIA MALAYI FUSION PROTEINS IN JIRDS, MERIONES UNGUICULATUS. J. Yates,\* J. Mooradian, C. Werner and T.V. Rajan. Oakland University, Department of Biological Sciences, Rochester, MI; Albert Einstein College of Medicine, Bronx, NY.
- 253 EICOSANOID FORMATION FROM ARACHIDONIC ACID BY MICROFILARIAE OF BRUGIA MALAYI: ENHANCEMENT BY HUMAN PLATELETS. L.X. Liu\* and P.F. Weller. Beth Israel Hospital, Harvard Medical School, Boston, MA.
- 254 MONOCLONAL ANTIBODIES SPECIFIC FOR BRUGIA PAHANGI TUBULIN. N.I. Bughio,\* G.M. Faubert and R.K. Prichard. Institute of Parasitology, McGill University, Montreal, CANADA.
- 255 THE USE OF CELL-CONDITIONED MEDIUM FOR THE IN VITRO CULTURE OF ONCHOCERCA SPP. LARVAE (NEMATODA: FILARIOIDES). M.S. Cupp,\* E.W. Cupp and C. Pouloupoulou. Department of Entomology, University of Arizona, Tucson, AZ.
- 256 CHARACTERIZATION OF THE THIOL-DEPENDENT DEVELOPMENTAL RESPONSE BY MICROFILARIAE OF ONCHOCERCA LIENALIS AND DIROFILARIA IMMITIS IN VITRO. R.J. Pollack,\* J.B. Lok and J.J. Donnelly. University of Pennsylvania, Philadelphia, PA.
- 257 GENETIC CONTROL OF FILARIAL WORM DEVELOPMENT IN DEFINED STRAINS OF AEDES AEGYPTI MOSQUITOES. A.R. Wattam,\* B.T. Beerntsen, J.M. Aiken and B.M. Christensen. University of Wisconsin, Madison, WI.
- 258 PHYSIOCHEMICAL DIFFERENCES IN SUSCEPTIBLE AND REFRACTORY STRAINS OF AEDES AEGYPTI (L.) TO INFECTION OF DIROFILARIA IMMITIS. J.K. Nayar,\* T.J. Bradley, J.W. Knight and F. Mahmood. Florida Medical Entomology Laboratory, IFAS - University of Florida, Vero Beach, FL; Department of Developmental and Cell Biology, University of California, Irvine, CA.

- 259 COMPETENCE OF CERTAIN COMMON EGYPTIAN MOSQUITO SPECIES FOR WUCHERERIA BANCROFTI. B.A. Soliman,\* A.M. Gad, A.A. Shoukry and S.M. El Said. Research and Training Center on Vectors of Diseases, Ain Shams University, Cairo, EGYPT.

SCHISTOSOMIASIS

- 260 PRODUCTION AND CHARACTERIZATION OF THREE RAT ANTI-MOUSE EOSINOPHIL MONOCLONAL ANTIBODIES. D. Gold,\* W.E. Secor, G.S. Henderson, S.J. Stewart and D.G. Colley. Vanderbilt University School of Medicine and Veterans Administration Medical Center, Nashville, TN.
- 261 NATURAL MOLLUSCICIDES FROM EGYPTIAN WILD HERBS. A.A.I. Elmagdoub,\* M.F. El-Sawy, J.B. Malone and S.A. Barker. College of Agriculture, and High Institute of Public Health, Alexandria University, EGYPT; School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.
- 262 SEGREGATION OF BULINUS TRUNCATUS AND HELISOMA DURYI. A COMPETITOR OF SCHISTOSOME INTERMEDIATE HOSTS, IN AN EGYPTIAN CANAL HABITAT. J.J. Sullivan,\* E. Ruiz-Tiben and M. Habib. Centers for Disease Control, Atlanta, GA; Qalyub Center for Field and Applied Research, MOH, EYPGT.
- 263 A MOLLUSCICIDE ISOLATED FROM THE PLANT TETRAPLEURA TETRAPTERA. O.D. Ngassapa,\* T.O. Henderson, N.R. Farnsworth, C.W.W. Beecher and G.L. Doye. Program for Collaborative Research in Pharm. Sci., and Department of Biological Chemistry, University of Illinois at Chicago, IL; Department of Pharmacology, University of Ghana Medical School, Accra, GHANA.
- 264 THE COUNTERIMMUNOELECTROPHORESIS (CIEP) FOR THE RAPID DIAGNOSIS OF FASCIOLIASIS AND SCHISTOSOMIASIS IN PATIENTS WITH PROLONGED FEVER AND EOSINOPHILIA. E.M. Mikhail,\* Z. Farid, F.G. Youssef and N.S. Mansour. United States Naval Medical Research Unit No. 3, Cairo, EGYPT.
- 265 SEROLOGICAL EVIDENCE OF THE APPLICABILITY OF ELISA IN BLOOD SPOTS ON FILTER PAPERS FOR THE CLINICAL STAGING OF HUMAN SCHISTOSOMIASIS MANSONI. K.A. Kamal,\* A. El-Said, H. Hamdto, S. Rashed and H. Shaheen. United States Naval Medical Research Unit No. 3, Cairo, EGYPT; Benha Medical School, Benha, EGYPT.
- 266 SEROLOGICAL DIFFERENCE BETWEEN ACUTE AND CHRONIC SCHISTOSOMA MANSONI IN RESPONSE TO KEYHOLE LIMPET HEMOCYANIN (KLH). M.M. Mansour,\* P. Omer-Ali, Z. Farid, A.J.G. Simpson and J.N. Woody. United States Naval Medical Research Unit No. 3, Cairo, EGYPT; National Institute for Medical Research, London, UNITED KINGDOM.
- 267 ATTRITION OF ADULT SCHISTOSOMA MANSONI AND A/J MICE. M.D. Maloney,\* G.C. Coles and L.H. Semprevivo. University of Massachusetts, Amherst, MA.

#### OTHER HELMINTHS

- 268 IDENTIFICATION OF CHEMICAL STIMULI FOR THE PENETRATION OF NECATOR AMERICANUS THIRD STAGE LARVAE IN VITRO. A.A. Siddiqui,\* A.C. Fusco and B. Salafsky. Department of Biomedical Sciences, University of Illinois College of Medicine, Rockford, IL.
- 269 IN VITRO ASSAY FOR RESUMPTION OF DEVELOPMENT IN INFECTIVE LARVAE (L3) OF SOIL-BORNE, PARASITIC NEMATODES. J.M. Howdon.\* University of Pennsylvania, Philadelphia, PA.
- 270 RADIOACTIVE LABELLING OF STRONGYLOIDES STERCORALIS BY FEEDING A SE-75 LABELLED METHIONINE-AUXOTROPHIC ESCHERICHIA COLI TO RHABDITIFORM LARVAE. L.M. Aikens\* and G.A. Schad. University of Pennsylvania, Philadelphia, PA.
- 271 INHIBITION OF THE COMPLEMENT CASCADE BY ANTIGEN B OF TAENIA SOLIUM. M. Rodriguez,\* A. Torre-Blanco, K. Willms and J.P. Lacllette. Department of Immunology, Instituto de Investigaciones Biomedicas, Facultad de Ciencias, UNAM, MEXICO D.F.
- 272 SEROLOGICAL DIAGNOSIS OF CYSTICERCOSIS USING AN ANTIGEN ISOLATED FROM TAENIA HYDATIGENA CYST FLUID. E.G. Hayunga,\* M.P. Sumner, M.L. Rhoads, K.D. Murrell, R.H. Fetterer and R.S. Isenstein. Serology Branch, Pathology and Epidemiology Division, Food Safety and Inspection Service; Helminthic Disease Laboratory, Livestock and Poultry Science Institute, Agricultural Research Service; Office of Area Director, ARC.
- 273 CHARACTERIZATION OF SECRETORY/EXCRETORY PRODUCTS OF SALINE- INCUBATED TOXOCARA CANIS LARVAE. E.J. Villanova\* and W.J. Kozek. Department of Microbiology and Medical Zoology, Medical Sciences Campus, University of Puerto Rico, San Juan, PUERTO RICO
- 274 PULMONARY IMMUNE RESPONSES TO NIPPOSTRONGYLUS BRASILIENSIS INFECTION IN RATS. K. Ramaswamy\* and D. Befus. University of Calgary, Calgary, CANADA.
- 275 COMPARATIVE ANALYSIS BY FLOW CYTOMETRY OF T CELL SUBSETS IN LUNGS AND SPLEENS OF MICE INFECTED WITH TOXOCARA CANIS. S.G. Kayes,\* M.B. Stober and R.B. Hester. Department of Anatomy and Cell Biology and Flow Cytometry Laboratory, College of Medicine, University of South Alabama, Mobile, AL.

#### TROPICAL VETERINARY MEDICINE

- 276 ANTIBODY RESPONSE IN WHITE-FOOTED MICE (PEROMYSCUS LEUCOPUS) EXPERIMENTALLY INOCULATED WITH BORRELIA BURGDORFERI, THE LYME DISEASE SPIROCHETE. T.G. Schwan,\* K.K. Kime and J.E. Coe. Laboratory of Pathobiology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT.
- 277 COMPARATIVE ANTIGENIC PROFILES OF EHRlichia CANIS, E. SENNETSU, E. EQUI, AND E. RISTICII. M.B. Nyindo, I. Kakoma, C.J. Holland,\* R. Hansen and M. Ristic. International Centre for Insect Physiology and Ecology, Nairobi, KENYA; University of Illinois, College of Veterinary Medicine, Urbana, IL.

- 278 AN IgM-SPECIFIC ANTIBODY-CAPTURE ELISA FOR RIFT VALLEY FEVER DIAGNOSIS IN SHEEP, CATLE AND GOATS. T.G. Ksiazek,\* J.M. Meegan, J.C. Morrill and C.J. Peters. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.
- 279 PATHOGENESIS OF COWDRIA RUMINANTIUM INFECTION IN GOATS, AS DETECTED THROUGH THE USE OF IMMUNOHISTOCHEMISTRY. C.C. Brown.\* Plum Island Animal Disease Center, United States Department of Agriculture, Agriculture Research Service, Greenport, NY.
- 280 TRANSMISSION ELECTRON MICROSCOPY OF MACROPHAGE CULTURES FROM COWDRIA RUMINANTIUM-INFECTED CATTLE. S.P. Sahu.\* Foreign Animal Disease Diagnostic Laboratory, APHIS, USDA, Greenport, NY.
- 281 DEMONSTRATION AND CHARACTERIZATION OF PROTEASE ACTIVITY IN RICKETTSIAL SPECIES OF THE SPOTTED FEVER GROUP. J.I. Herrero, A. Alam and D.H. Walker. University of Texas Medical Branch, Galveston, TX.
- 282 A MOUSE LETHAL DOSE ASSAY FOR DETECTION OF COWDRIA RUMINANTIUM (KWANYANGA STRAIN) IN GOATS AND TICKS. R.G. Endris, T.M. Haslett,\* E.F. Birnie and W.R. Hess. USDA-ARS, Plum Island Animal Disease Center, Greenport, NY.
- 283 EXTRAPOLATION OF A LOUISIANA CLIMATE BASED FORECASTING SYSTEM FOR FASCIOLA HEPATICA TO RAINFALL-DEPENDENT PASTURE ZONES OF FLORIDA AND EXAS. J.B. Malone,\* R.A. Riggelman and R.A. Muller. Louisiana State University, Baton Route, LA.

#### LEISHMANIASIS AND TRYPANOSOMIASIS

- 284 CUTANEOUS LEISHMANIASIS IN THE GUATEMALAN ARMY. M. Grog1,\* P.W. Kelley, W.R. Ballou, J.D. Berman, D. Gordon, E. Rowton and R.D. Kreutzer. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; Youngstown State University, Youngstown, OH.
- 285 CUTANEOUS LEISHMANIASIS IN HONDURAS CAUSED BY L. DONOVANI CHAGASI. C. Ponce,\* E. Ponce, A. Morrison, A. Cruz and F. Neva. Ministry of Health, Tegucigalpa, HONDURAS; United States Peace Corps, HONDURAS; Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.
- 286 GEOGRAPHIC DISTRIBUTION AND CLINICAL PRESENTATION OF LEISHMANIASIS IN PERU. E.D. Franke,\* C. Lucas, A. Tovar, A. Tejada and F.S. Wignall. United States Naval Medical Research Institute Detachment, Lima, PERU; Medicina Tropical, University San Marcos, Lima, PERU.
- 287 CLINICAL AND EPIDEMIOLOGIC STUDIES OF MUCOCUTANEOUS LEISHMANIASIS (MCL) DUE TO LEISHMANIASIS BRAZILIENSIS PANAMENSIS IN PANAMA. R.E. Saenz,\* R. Mata, G. de Rodriguez, A.M. de Vasquez, H. Paz, A. de Perez and L.A. Quiel. Gorgas Memorial Laboratory, PANAMA.
- 288 SCREENING FOR LEISHMANIASIS WITH A TOTAL BLOOD MICRO LYMPHOCYTE PROLIFERATION ASSAY IN A GROUP OF CHILDREN IN JERICHO. S. Frankenburg,\* K. Jaber, R. Alvarado, L. Schnur and C. Enk. The Kuvim Centre for the Study of Infectious and Tropical Diseases, Jerusalem, ISRAEL; Public Health Department, Jericho, ISRAEL.

- 289 LEISHMANIA INFANTUM ISOLATED FROM DOGS IN EL AGAMY, ALEXANDRIA, EGYPT. M.G. Shehata,\* B.M. El Sawaf, S.M. El Said, S. Doha, J. Dereure, F. Pratlong and J.A. Rioux. Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, EGYPT; Laboratoire d'Ecologie medicale et Pathologie parasitaire, Faculte de Medecine, Universite de Montpellier, FRANCE.
- 290 DEVELOPMENT OF LEISHMANIA MAJOR IN PHLEBOTOMUS DUBOSCOI AND SERGENTOMYIA SCHWETZI. P.G. Lawyer,\* P. Ngumbi, C. Anjili, S. Odongo, Y. Mebrahtu, J. Tighure, D.K. Koech and C.R. Roberts. Kenya Medical Research Institute and United States Army Medical Research Unit, Nairobi, KENYA.
- 291 TRYPANOSOMA CRUZI ANTIGEN RECOGNITION BY SERA FROM GUATEMALA. V.L. Matta\* and J.E. Deas. Louisiana State University Medical Center, New Orleans, LA.
- 292 EXPERIMENTAL CHRONIC CHAGAS' DISEASE IN DOGS. S.C. Barr,\* R.A. Holmes, S.P. Schmidt, C. Brown, V.A. Dennis and T.R. Klein. School of Veterinary Medicine, Louisiana State University, Baton Route, LA.
- 293 MYOCARDIAL ADENYLATE CYCLASE ACTIVITY IS REVERSED BY VERAPAMIL IN ACUTE CHAGAS' DISEASE. S.A. Morris,\* L.M. Weiss, S.M. Factor, J.P. Bilezikian, H.B. Tanowitz, M. Wittner. Albert Einstein College of Medicine, New York, NY.
- 294 VERAPAMIL MODIFIES MURINE CHAGASIC CARDIOMYOPATHY. L.M. Weiss,\* S.A. Morris, S.M. Factor, V. Braunstein, H.B. Tanowitz and M. Wittner. Albert Einstein College of Medicine, Bronx, NY.
- 295 RAPID, COMPUTER-ASSISTED, IN VITRO, MICRO-METHOD FOR QUANTITATIVE ASSESSMENT OF LEISHMANIA SPP. RESISTANCE TO PENTAVALENT ANTIMONIALS. J.E. Jackson\* and J.D. Tally. Department of Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.

WEDNESDAY MORNING - DECEMBER 7

CRAIG LECTURE

11:00 AM - 12:00 Noon

Regency

- 296 AEDES AEGYPTI AND AEDES AEGYPTI-BORNE DISEASE CONTROL IN THE 1990S: TOP DOWN OR BOTTOM UP? Duane J. Gubler. Chief, Dengue Branch, and Director of the San Juan Laboratories, Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, San Juan, PUERTO RICO.

## WEDNESDAY AFTERNOON - DECEMBER 7

## PAUL C. BEAVER SYMPOSIUM - PARASITOLOGY THEN AND NOW

1:30 PM - 5:00 PM

Palladian

Chairpersons: L.R. Ash, R. Jung and E.A. Ottesen

<u>Time</u>	<u>Abst</u>	
1:30	297	PAUL BEAVER: AN OVERVIEW OF 60 YEARS OF RESEARCH. R. Jung. Tulane University School of Medicine, New Orleans, LA.
1:50	298	FILARIASIS THEN. T.C. Orihel. Tulane University School of Medicine, New Orleans, LA.
2:00	299	FILARIASIS NOW. E.A. Ottesen. Laboratory of Clinical Investigation, National Institutes of Health, Bethesda, MD.
2:20	300	LARVA MIGRANS THEN. L.R. Ash. UCLA School of Public Health, Los Angeles, CA.
2:30	301	LARVA MIGRANS NOW. P.M. Schantz. Centers for Disease Control, Bethesda, MD.
3:00		COFFEE BREAK.
3:30	302	HOOKWORM THEN. M.D. Little. Tulane University School of Medicine, New Orleans, LA.
3:40	303	HOOKWORM NOW. G.A. Schad. University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA.
4:00	304	AMEBIASIS THEN. A. D'Alessandro. Tulane University School of Medicine, New Orleans, LA.
4:10	305	AMEBIASIS NOW. J.I. Ravdin. University of Virginia, Charlottesville, VA.
4:30		DISCUSSION.

WEDNESDAY AFTERNOON - DECEMBER 7

29TH ANNUAL OPEN MEETING OF THE AMERICAN  
COMMITTEE ON ARTHROPOD-BORNE VIRUSES

BUSINESS MEETING

1:30 PM - 2:30 PM

Diplomat

Chairperson: L.D. Kramer

2:15 WILLIAM F. SCHERER AWARD.

SCIENTIFIC SESSION: VIRAL PATHOGENESIS AND VIRAL PERSISTENCE IN NATURE

2:30 PM - 5:30 PM

Diplomat

Chairperson: C.J. Peters

Time    Abst

- 2:30                    INTRODUCTION. C.J. Peters. United States Army Medical  
Research Institute of Infectious Diseases, Ft. Detrick, MD.
- 2:35    306    PATHOGENICITY AND PERSISTENCE OF THOGOTO VIRUS, A CANDIDATE  
MEMBER OF THE ORTHOMYXOVIRIDAE. P. Nuttall, L.D. Jones, C.R.  
Davies, T. Booth and D. Staunton. National Environmental  
Research Council, Institute of Virology, Oxford, UNITED  
KINGDOM.
- 3:05                    COFFEE BREAK
- 3:35    307    ECOLOGIC FACTORS IN LASSA VIRUS TRANSMISSION. J.B.  
McCormick. Centers for Disease Control, Atlanta, GA.
- 4:05    308    MAINTENANCE AND TRANSMISSION OF HANTAVIRUSES IN RODENT  
POPULATIONS. J.E. Childs, J.W. LeDuc, G.E. Glass and G.W.  
Korch. Johns Hopkins School of Hygiene, Baltimore, MD; United  
States Army Medical Research Institute of Infectious Diseases,  
Ft. Detrick, MD.
- 4:35    309    RIFT VALLEY FEVER EPIDEMIC IN MAURITANIA: EPIDEMIOLOGY AND  
SPECULATIONS ON ORIGINS. J.P. Digoutte. Institute Pasteur,  
Dakar, SENEGAL.
- 5:00    310-    EPIDEMIC REPORTS  
         315

WEDNESDAY AFTERNOON - DECEMBER 7

SCIENTIFIC SESSION O: MALARIA - CHEMOTHERAPY

1:30 PM - 5:00 PM

Empire

Chairpersons: B. Schuster and T.E. Wellems

<u>Time</u>	<u>Abst</u>	
1:30	316	REVERSAL OF CHLOROQUINE RESISTANCE IN <u>PLASMODIUM FALCIPARUM</u> WITH CALCIUM ANTAGONISM IN THE NANOMOLAR RANGE. A.J. Bitonti,* P.P. McCann and A. Sjoerdsma. Merrell Dow Research Institute, Cincinnati, OH.
1:45	317	CHLOROQUINE RESISTANCE IN <u>PLASMODIUM FALCIPARUM</u> AND MULTIDRUG-RESISTANT CHINESE HAMSTER OVARY (CHO) CELLS. B.L. Herwaldt,* I.Y. Gluzman, D.J. Krogstad, P.H. Schlesinger, V. Ling, S.A.W. Fuqua, A.K. Tandon and W.L. McGuire. Washington University, St. Louis, MO; Ontario Cancer Institute, Princess Margaret Hospital, Toronto, CANADA; University of Texas Health Science Center, San Antonio, TX.
2:00	318	REVERSAL OF MEFLOROQUINE RESISTANCE IN <u>PLASMODIUM FALCIPARUM</u> IN <u>VITRO</u> . D.E. Kyle,* W.K. Milhous and A.M.J. Oduola. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
2:15	319	CYCLOGUANIL AND SULFAMETHOXAZOLE EXHIBIT ENHANCED <u>IN VITRO</u> ACTIVITY OVER SULFADOXINE AND PYRIMETHAMINE. W.K. Milhous,* A.M.J. Oduola, D.E. Kyle, P.F. Pierce, L. Gerena, K. Canfield, B.G. Shuster and C.J. Canfield. Walter Reed Army Institute of Research, Walter Reed Army Medical Center and Georgetown University Medical Center, Washington, DC; Pharmaceutical Systems Inc., Gaithersburg, MD.
2:30	320	PROTECTION AGAINST MALARIA IN MICE BY NUTRITIONAL MANIPULATION OF HOST ANTIOXIDANT STATUS. O.A. Levander,* A.L. Ager, Jr., V.C. Morris and R.G. May. USDA, Human Nutrition Research Center, Beltsville, MD; Center Tropical Parasitic Diseases, University of Miami, Miami, FL.
2:45	321	IMMUNOLOGIC CHARACTERIZATION AND MOLECULAR CLONING OF FILARIAL ANTIGENS. D.L. Ellenberger,* N.J. Pieniazek, M.L. Eberhard, R.C. Lowrie, Jr. and P.J. Lammie. Louisiana State University Medical Center of New Orleans, LA; Centers for Disease Control, Atlanta, GA; Delta Regional Primate Center, Covington, LA; ICIDR Program, Tulane University, New Orleans, LA.
3:00		COFFEE BREAK
3:30	322	SPORONTICIDAL ACTIVITY OF BRUCEINE A AGAINST A THAI ISOLATE OF <u>PLASMODIUM FALCIPARUM</u> . K. Pavanand, J. Sattabongkot, N. Lutthiwongsakorn, M. Rasameesoraj, K. Yongvanitichit and H.K. Webster. USAMC, AFRIMS, Bangkok, THAILAND.

- 3:45 323 RADICAL CURATIVE PROPERTIES OF WR238605. G.E. Heisey,\* W.K. Milhous, P. Hansuklarita, A.D. Theoharides, B.G. Schuster and D.E. Davidson, Jr. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; Armed Forces Research Institute of Medical Sciences, Bangkok, THAILAND.
- 4:00 324 MEFLOQUINE PROPHYLAXIS - LACK OF ACCUMULATION ON 250 MG WEEKLY DOSING. E.F. Boudreau, L. Fleckenstein and L.W. Pang. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; the Armed Forces Research Institute of Medical Science, Bangkok, THAILAND.
- 4:15 325 MEFLOQUINE THERAPY IN CHILDREN UNDER FIVE IN MALAWI: CORRELATION OF BLOOD DRUG CONCENTRATION WITH PARASITOLOGIC FAILURES. L. Slutsker,\* C.O. Khoromana, D. Payne, L. Ptchen and D.L. Heymann. Malaria Branch and Control Technology Branch, Centers for Disease Control, Atlanta, GA; World Health Organization, Geneva, SWITZERLAND; Ministry of Health, MALAWI.
- 4:30 326 ANTIMALARIAL DRUG USE PRACTICES AND PREFERENCES IN GUATEMALA. T.K. Ruebush II,\* S.C. Weller, R.E. Klein, H. Godoy and A. Mendez. Medical Entomology Research and Training Unit/GUATEMALA; Centers for Disease Control, Atlanta, GA; Department of Medicine, University of Pennsylvania, Philadelphia, PA; Division of Malaria, Ministry of Health and University del Valle, Guatemala City, GUATEMALA.
- 4:45 327 THE RESPONSE OF NIGERIAN CHILDREN WITH PLASMODIUM FALCIPARUM TO CHLOROQUINE AND TO SULFADOXINE/PYRIMETHAMINE. J.G. Breman,\* O.J. Ekanem, J.S. Weisfeld, L.A. Salako, B.L. Nahlen, E.N.U. Ezedinahi, O. Walker, O.J. Laoye and K. Hedberg. Malaria Branch and International Health Program Office, Centers for Disease Control, Atlanta, GA; Ministry of Health, NIGERIA.

WEDNESDAY AFTERNOON - DECEMBER 7

ASTVM ANNUAL MEETING: EHRLICHIOSIS SYMPOSIUM

1:30 PM - 5:30 PM

Executive

Chairpersons: I. Kakoma and J.C. Williams

Time    Abst

- 1:30 328 THE HISTORICAL BACKGROUND AND GLOBAL IMPORTANCE OF EHRLICHIOSIS. D.L. Huxsoll, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.
- 1:50 329 ADVANCES IN THE IN VITRO CULTIVATION OF EHRLICHIAE. M.B.A. Nyindo and C.J. Holland, ICIPE, Nairobi, KENYA; the University of Illinois, Urbana, IL.

- 2:10 330 BIOLOGIC AND PATHOGENIC PROPERTIES OF EHRlichia RISTICII.  
C.J. Holland, University of Illinois, Urbana, IL.
- 2:30 331 ANTIGENIC PROPERTIES OF THE EHRlichIAE AND OTHER  
RICKETTSIACEAE. G.A. Dasch, E. Weiss and J.C. Williams.  
Naval Medical Research Institute, Bethesda, MD; United States  
Army Medical Research Institute of Infectious Diseases, Fort  
Detrick, Frederick, MD.
- 2:50 COFFEE BREAK
- 3:30 332 BIOLOGICAL PROPERTIES OF EHRlichIA: SUBSTRATE UTILIZATION AND  
ENERGY METABOLISM. E. Weiss, G.A. Dasch, J.C. Williams, and  
Y.H. Kang. Emeritus Scientist, NMRI, Bethesda, MD; United  
States Army Medical Research Institute of Infectious Diseases,  
Ft. Detrick, MD.
- 3:50 333 EHRlichIOSIS IN NONHUMAN PRIMATES. E.H. Stephenson,  
Virginia-Maryland Regional College of Veterinary Medicine,  
Blacksburg, MD.
- 4:10 334 HUMAN EHRlichIOSIS; RECOGNITION AND CHARACTERIZATION OF THE  
SYNDROME. D.B. Fishbein, J. Dawson, M. Mebus and A. Kemp.  
Centers for Disease Control, Atlanta, GA.
- 4:30 335 RECENT RESEARCH FINDINGS ON COWDRIOSIS. J.D. Bezuidenhout.  
Onderstepoort, RSA.
- 4:50 336 CURRENT STRATEGIES AND PROGRESS IN RESEARCH ON EHRlichIOSIS.  
M. Ristic. College of Veterinary Medicine, University of  
Illinois, Urbana, IL.
- 5:10 337 EVOLUTIONARY HISTORY OF CHLAMYDIAE: ANSWERS FOR SOME OLD  
QUESTIONS, NO ANSWERS FOR SOME NEW ONES. J.W. Moulder.  
Emeritus Professor, University of Chicago, IL.

WEDNESDAY AFTERNOON - DECEMBER 7

SCIENTIFIC SESSION P: ERYTHROCYTIC ANTIGENS AND IMMUNOLOGY

1:30 PM - 5:00 PM

Congressional

Chairpersons: N. Brown and C. Long

Time    Abst

- 1:30 338 BIOLOGICAL PROPERTIES OF T CELL LINES TO THE MURINE MALARIAL  
PARASITE PLASMODIUM CHABAUDI ADAMI. J. Melancon-Kaplan, D.M.  
Russo and W.P. Weidanz. Hahnemann University, Philadelphia,  
PA.
- 1:45 339 MEMBRANE-ASSOCIATED ANTIGENS OF BLOOD STAGES OF PLASMODIUM  
BRASILIANUM, A QUARTAN MALARIA PARASITE. A.H. Cochrane,\* Y.  
Matsumoto, K.K. Kamboj, M. Maracic, R.S. Nussenzweig and M.  
Aikawa. Department of Medical and Molecular Parasitology, New  
York University School of Medicine, New York, NY; Institute of  
Pathology, Case Western Reserve University, Cleveland, OH.

- 2:00 340 A MONOCLONAL ANTIBODY AGAINST A 50 KD PROTEIN IN MAURER'S CLEFTS OF PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES REACTS WITH HUMAN LEUKOCYTES. F.W. Klotz,\* A. Szarfman, E. Rock, M. Aikawa, R. Howard, S. Cohen and L. Miller. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; Naval Medical Research Institute, USUHS and National Institutes of Health, Bethesda, MD; Case Western Reserve University, Cleveland, OH.
- 2:15 341 CHARACTERIZATION OF A PLASMODIUM FALCIPARUM ERYTHROCYTE BINDING ANTIGEN USING AN AFFINITY PURIFIED MONOSPECIFIC ANTIBODY REAGENT. P.A. Orlandi,\* B.K.L. Sim, J.D. Haynes, M. Zegans and J.D. Chulay. Department of Immunology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 2:30 342 ISOLATION AND CHARACTERIZATION OF THE GENE ENCODING A PLASMODIUM FALCIPARUM ERYTHROCYTE BINDING ANTIGEN. B.K.L. Sim, P.A. Orlandi, G.S. Leppert, J.D. Haynes, D. Camus and J.D. Chulay. Department of Immunology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 2:45 343 FURTHER CHARACTERIZATION OF PLASMODIUM FALCIPARUM ANTIGENS PRESENT IN IMMUNE CLUSTERS OF MEROZOITES. G. Watt,\* J.A. Lyon and J.D. Chulay. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 3:00 COFFEE BREAK
- 3:30 344 PEPTIDASES FROM SCHIZONTS OF PLASMODIUM FALCIPARUM CULTURED IN VITRO. M. Nwagwu,\* J.D. Haynes, P.A. Orlandi, A. Meierovics and J.D. Chulay. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.
- 3:45 345 IMMUNOLOGICAL STUDIES OF A YEAST RECOMBINANT POLYPEPTIDE BASED ON THE AMINO TERMINAL REGION OF P. FALCIPARUM GP195. S.P. Chang,\* G.S.N. Hui, P. Barr, H. Gibson, K. Kramer, A. Kato and W.A. Siddiqui. Department of Tropical Medicine and Medical Microbiology, University of Hawaii, Honolulu, HI; Chiron Corporation, Emeryville, CA.
- 4:00 346 LACK OF CORRELATION BETWEEN IMMUNITY TO MALARIA AND IN VITRO PRODUCTION OF GAMMA-INTERFERON BY T LYMPHOCYTES CULTURED IN THE PRESENCE OF PLASMODIUM FALCIPARUM ANTIGEN. C. Chizzolini,\* G. Grau, A. Geino and D. Schrijvirs. Centre International de Recherche Medicale, Franceville, GABON; and World Health Organization International Research and Training Center, University of Geneva, SWITZERLAND.
- 4:15 347 INCREASED LEVELS OF SOLUBLE INTERLEUKIN 2 RECEPTORS IN PLASMODIUM FALCIPARUM MALARIA. P. Nguyen-Dinh\* and A.E. Greenberg. Malaria Branch, Centers for Disease Control, Atlanta, GA.

- 4:30 348 CHANGES IN PLASMA TUMOR NECROSIS FACTOR ALPHA (TNF) AND INTERLEUKIN-1 BETA (IL-1) FOLLOWING CHALLENGE OF NONIMMUNE OR IMMUNE HUMAN VOLUNTEERS WITH PLASMODIUM VIVAX. C. Munoz, J.R. Murphy, S. Baqar, J. Davis, D.A. Herrington and M.M. Levine. Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD.
- 4:45 349 IMMUNOLOGICAL STUDIES OF THE EFFECTS OF PLASMODIUM FALCIPARUM HEMOZOIN ON NORMAL HUMAN MONONUCLEAR CELLS. P. Goldie,\* F.T. Valentine and J.P. Vanderberg. Department of Medical and Molecular Parasitology, Department of Medicine, Infectious Diseases and Immunology Division, New York University Medical Center, New York, NY.

THURSDAY MORNING - DECEMBER 8

SCIENTIFIC SESSION Q: ARBOVIROLOGY - MOLECULAR BIOLOGY AND VACCINE DEVELOPMENT

8:00 AM - 11:00 AM

Ambassador

Chairpersons: T.P. Monath and C.S. Schmaljohn

- | <u>Time</u> | <u>Abst</u> |                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|-------------|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 8:00        | 350         | SYNTHETIC PEPTIDES DERIVED FROM THE DEDUCED AMINO ACID SEQUENCE OF THE E-GLYCOPROTEIN OF MURRAY VALLEY ENCEPHALITIS VIRUS ELICIT ANTI-VIRAL IMMUNITY. J.T. Roehrig, A.R. Hunt, A.J. Johnson and R.A. Hawkes. Centers for Disease Control, Fort Collins, CO; School of Microbiology, University of New South Wales, Kensington, New South Wales, AUSTRALIA.                                                                                                  |
| 8:30        | 351         | ANTIGENICITY AND IMMUNOGENICITY OF RIFT VALLEY FEVER VIRUS GLYCOPROTEINS EXPRESSED BY BACULOVIRUS RECOMBINANTS. W. Ennis,* A. Schmaljohn, L. Rasmussen and C. Schmaljohn. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.                                                                                                                                                                                            |
| 8:45        | 352         | USE OF DENGUE VIRUS STRUCTURAL PROTEINS AND NONSTRUCTURAL PROTEIN NS1 PRODUCED BY RECOMBINANT BACULOVIRUS FOR IMMUNIZATION AGAINST DENGUE VIRUS INFECTION. Y.M. Zhang, D.R. Dubois, K.H. Eckels, R.M. Chanock and C.J. Lai.* Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD; Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC. |
| 9:00        | 353         | RECOMBINANT VACCINIA VIRUSES EXPRESSING DENGUE 4 STRUCTURAL OR NON-STRUCTURAL PROTEINS PROTECT MICE AGAINST LETHAL CHALLENGE. M. Bray,* B.T. Zhao, B. Falgout, K.H. Eckels and C.J. Lai. Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD; Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC.                                     |

- 9:15 354 EXPRESSION OF THE M AND THE S GENOME SEGMENTS OF HANTAAVIRUS BY VACCINIA AND BACULOVIRUS RECOMBINANTS. C.S. Schmaljohn, A.L. Schmaljohn and J.M. Dalrymple. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.
- 9:30 355 PROTECTION AGAINST DENGUE AFFORDED BY IMMUNIZATION WITH YELLOW FEVER 17D. T.P. Monath,\* C.B. Cropp, W.A. Short, M. Chu, C.J. Mitchell and D.J. Gubler. Division of Vector-Borne Viral Diseases, Centers for Disease Control, Fort Collins, CO.
- 9:45 356 IMMUNIZATION WITH LIVE-ATTENUATED DENGUE TYPE 4 (341750 CARIB) VIRUS VACCINE. C.H. Hoke,\* F.J. Malinoski, R. McN. Scott, K.H. Eckels, D.R. Dubois, T.E. Simms, J. McCown, P. Summers, D.S. Burke and W.H. Bancroft. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.
- 10:00 COFFEE BREAK
- 10:30 357 EVALUATION OF A LIVE, ATTENUATED, VENEZUELAN EQUINE ENCEPHALOMYELITIS VACCINE (TC-83). N.A. Popovic,\* D.D. Oland and T.M. Cosgriff. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.
- 10:45 358 FURTHER STUDIES ON THE SAFETY AND PROTECTIVITY OF A MUTAGENIZED RIFT VALLEY FEVER VIRUS IMMUNOGEN. J.C. Morrill,\* L. Carpenter, D. Taylor, H. Ramsburg, J. Dalrymple, H.W. Lupton and C.J. Peters. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

THURSDAY MORNING - DECEMBER 8

TROPICAL MEDICINE COMMEMORATIVE FUND LECTURE:

11:00 AM

Ambassador

Time    Abst

- 11:00 359 PROGRESS IN THE TREATMENT AND PREVENTION OF ARGENTINE HEMORRHAGIC FEVERS. Julio I. Maiztegui. Director, Instituto Nacional de Estudio sobre Virosis Hemorragicas, Pergamino, ARGENTINA.

THURSDAY MORNING - DECEMBER 8

SCIENTIFIC SESSION R: CLINICAL TROPICAL MEDICINE

8:15 AM - 12:00 Noon

Diplomat

Chairpersons: D. Parenti and H. Tanowitz

<u>Time</u>	<u>Abst</u>	
8:15	360	<u>CAMPYLOBACTER ENTERITIS ASSOCIATED WITH DOXYCYCLINE PROPHYLAXIS FOR MALARIA IN THAILAND.</u> D.N. Taylor,* C. Pitarangsi and P. Echeverria. Armed Forces Research Institute of Medical Sciences, Bangkok, THAILAND.
8:30	361	<u>COST BENEFIT OF VACCINATION WITH Ty21a ORAL TYPHOID VACCINE IN PLAJU, INDONESIA.</u> N.H. Punjabi,* F.P. Paleologo, C.H. Simanjuntak, A.S. Dicky and H.M.P. Choesni. United States Naval Medical Research Unit-2 Detachment, NIHR&D Health Ministry, Pertamina Oil Co., INDONESIA.
8:45	362	<u>RIFT VALLEY FEVER EPIDEMIC IN MAURITANIA.</u> J.P. DiGoutte, A. Jouan, B. LeGuanno, O. Riou, B. Philippe, F. Adam, J. Meegan and C.J. Peter. Institut Pasteur, Dakar, SENEGAL; Hopital of Rosso, MAURITANIA; United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.
9:00	363	<u>CLINICAL AND EPIDEMIOLOGIC CHARACTERISTICS OF AFRICAN TRYPANOSOMIASIS IN AMERICAN TRAVELERS.</u> R.T. Bryan, H. Waskin, F. Richards, T. Bailey and D. Juranek. Parasitic Diseases Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.
9:15	364	<u>PLACEBO-CONTROLLED COMPARISON OF LOCAL HYPERTHERMIA AND GLUCANTIME (MEGLUMINE ANTIMONATE) IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS IN GUATEMALA.</u> T.R. Navin,* B.A. Arana, A.M. de Merida, F.E. Arana, E. Silva, J.L. Pozuelos and N. Levine. Medical Entomology Research and Training Unit/Guatemala, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.
9:30	365	<u>CYSTIC HYDATID DISEASE: NEED FOR A REAPPRAISAL OF ASPIRATION CYTOLOGY FOR DIAGNOSIS:</u> P.R. Hira,* H. Schweiki, L.G. Lindberg, I. Francis, Y. Shaheen, H. Leven and K. Behbehani. Health Sciences Center, Kuwait University, Safat, and Mubarek Hospital, Ministry of Public Health, KUWAIT.
9:45	366	<u>MALARIA PROPHYLAXIS WITH PROGUANIL/SULFONAMIDE IN THAILAND.</u> J.J. Karwacki, G.D. Shanks,* L.W. Pang, N. Limsomwong and P. Singharaj. Armed Forces Research Institute of Medical Sciences, APO San Francisco, CA.
10:00		COFFEE BREAK
10:30	367	<u>CHLOROQUINE-INDUCED PRURITUS.</u> A.U. Orjih,* M.D. Ene and D.J. Krogstad. University of Port Harcourt, NIGERIA; Washington University, St. Louis, MO.

- 10:45 368 EFFICACY AND SEVERE SIDE EFFECTS OF MEFLOROQUINE AGAINST FALCIPARUM MALARIA IN FRENCH TRAVELLERS. J. Le Bras, B. Rouveix, F. Simon, F. Bricaire and J.P. Coulade. National Reference Center for Chemosensitivity of Malaria and INSERM U13; Hop. Claude Bernard, Paris, FRANCE.
- 11:00 369 MALARIA PREVENTION IN TRAVELERS TO KENYA. H.O. Lobel,\* A.D. Brandling-Bennett, R. Steffen, P. Phillips-Howard and J.B.O. Were. Malaria Branch, Centers for Disease Control, Atlanta, GA; Kenya Medical Research Institute; NAIROBI; Institute of Preventive and Social Medicine, Zurich, SWITZERLAND; London School of Hygiene and Tropical Medicine, London, UNITED KINGDOM.
- 11:15 370 MORTALITY DUE TO IMPORTED PLASMODIUM FALCIPARUM MALARIA IN UNITED STATES TRAVELERS. 1959-1987. A.E. Greenberg\* and H.O. Lobel. Malaria Branch, Centers for Disease Control, Atlanta, GA.
- 11:30 371 REASSESSMENT OF BLOOD DONOR SELECTION CRITERIA FOR U.S. TRAVELERS TO MALARIOUS AREAS. B.L. Nahlen,\* S.E. Cannon and C.C. Campbell. Malaria Branch, Centers for Disease Control, Atlanta, GA.
- 11:45 372 THE SITES, SOUNDS AND SYMPTOMS OF DERMATOBIA HOMINIS: A STEREO SCAN, MOTION AND ELICITED HOST RESPONSE STUDY. M.E. Gordon.\* Yale School of Medicine, New Haven, CT.

THURSDAY MORNING, DECEMBER 8

SCIENTIFIC SESSION S: MALARIA - SPOROZOITES

8:15 AM - 12:00 Noon

Palladian

Chairpersons: A.H. Cochrane and J. Playfair

Time    Abst

- 8:15 373 IMMUNIZATION OF HUMAN VOLUNTEERS WITH A RECOMBINANT PLASMODIUM FALCIPARUM SPOROZOITE VACCINE, R32NS181. J.E. Egan,\* T.A. Cosgriff, G.F. Wasserman, J.F. Young, R.A. Wirtz, L. Schneider, M.R. Hollingdale, J.D. Chulay and W.R. Ballou. Departments of Immunology and Entomology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; Department of Medicine, United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; Biomedical Research Institute, Rockville, MD; Smith Kline and French Laboratories, Swedeland, PA.
- 8:30 374 ESTIMATE OF PLASMODIUM FALCIPARUM SPOROZOITE CONTENT OF ANOPHELES STEPHENSI USED TO CHALLENGE HUMAN VOLUNTEERS. J.R. Davis,\* J.R. Murphy, S. Baqar, A.H. Cochrane, F. Zavala, R.S. Nussenzweig and M.M. Levine. Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD.

- 8:45 375 SAFETY AND IMMUNOGENICITY OF CONJUGATE PLASMODIUM FALCIPARUM SPOOROZOITE VACCINES USING SYNTHETIC OR RECOMBINANT ANTIGENS COUPLED TO CHOLERA TOXIN AND PSEUDOMONAS TOXIN A. J.C. Sadoff, L.D. Loomis,\* J.U. Que, S.J. Cryz, R. Steffen, J.F. Young, E. Furer and W.R. Ballou. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; SSVI, Berne, SWITZERLAND; Smith Kline and French Laboratories, Swedeland, PA.
- 9:00 376 HLA-DR AND LYMPHOCYTE RESPONSIVENESS TO THE CIRCUMSPOROZOITE PROTEIN OF PLASMODIUM FALCIPARUM. A.E. Brown,\* D. Chandanayingyong and H.K. Webster. United States Army Medical Component, AFRIMS, Bangkok and Blood Bank, Siriraj Hospital, Bangkok, THAILAND.
- 9:15 377 PLASMODIUM FALCIPARUM CS VACCINES ELICIT ANTIBODIES IN HUMAN VOLUNTEERS THAT INHIBIT SPOOROZOITE INVASION OF HEPATOMA CELLS BUT ENHANCE INVASION OF HUMAN HEPATOCYTES. M.R. Hollingdale,\* D. Mazier, A. Appiah, P. Leland, T. Derks, W.R. Ballou, D.A. Herrington, S.H. Yap and J.P. Verhave. Biomedical Research Institute, Rockville, MD; CHU Pitie-Saltpetriere, Paris, FRANCE; University of Nijmegen Medical School, THE NETHERLANDS; Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; University of Maryland School of Medicine, Baltimore, MD.
- 9:30 378 PLASMODIUM FALCIPARUM SPOOROZOITES FROM MOSQUITOES PREVIOUSLY FED ANTI-NANP ANTIBODIES ARE NO LONGER NEUTRALIZED BY SERA FROM A HUMAN VOLUNTEER IMMUNIZED WITH R32TET32 VACCINE AND PROTECTED TO CHALLENGE. M.R. Hollingdale,\* A. Appiah, J. Vaughan and V.E. do Rosario. Biomedical Research Institute, Rockville, MD; School of Medicine, University of Maryland, Baltimore, MD.
- 9:45 379 HUMORAL AND CELLULAR IMMUNE RESPONSES IN VOLUNTEERS IMMUNIZED WITH IRRADIATED P. FALCIPARUM SPOOROZOITES. E.H. Nardin,\* D. Herrington, M. Levine, J. Murphy, J. Davis, V. Nussenzweig and R. Nussenzweig. Department of Medical and Molecular Parasitology, New York University School of Medicine and Center for Vaccine Development, University of Maryland, Baltimore, MD.
- 10:00 COFFEE BREAK
- 10:30 380 PRESENTATION OF PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN EPITOPES ON THE SURFACE OF RECOMBINANT HEPATITIS B SURFACE ANTIGEN TO ENHANCE IMMUNOGENICITY OF A SPOOROZOITE VACCINE. D.M. Gordon,\* T. Rutgers, A.M. Gathoye, W.R. Ballou, M. DeWilde and M. Rosenberg. Department of Immunology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC; Department of Molecular Genetics, Smith Kline-RIT and Smith Kline and French, Swedeland, PA.
- 10:45 381 PLASMODIUM FALCIPARUM SPOOROZOITES PROTECT MICE TO CHALLENGE WITH P. BERGHEI SPOOROZOITES. V.E. Rosario,\* J. Vaughan, S. Aley, G. Woollett and M.R. Hollingdale. Biomedical Research Institute, Rockville, MD; School of Medicine, University of Maryland, Baltimore, MD.

- 11:00 382 MAPPING OF T CELL EPITOPES WITHIN THE P. BERGHEI CS PROTEIN. P. Romero,\* J.P. Tam, J. Schlesinger, V. Nussenzweig, R.S. Nussenzweig and F. Zavaia. New York University School of Medicine, New York, NY; The Rockefeller University, New York, NY.
- 11:15 383 PROTECTION OF MICE AGAINST PLASMODIUM YOELII SPOROZOITE-INDUCED MALARIA BY PASSIVE TRANSFER OF A MONOCLONAL ANTIBODY TO A DEFINED EPITOPE. Y. Charoenvit,\* M. Sedegah, M. Leef, P. De la Vega, C. Cole, R.L. Beaudoin and S.L. Hoffman. Infectious Disease Department, Naval Medical Research Institute, Bethesda, MD; HPT, PAHO, Washington, DC.
- 11:30 384 COMPLETE PROTECTION AGAINST PLASMODIUM YOELII MALARIA BY IMMUNIZATION WITH A RECOMBINANT CS PROTEIN VACCINE. L.F. Yuan,\* M. Gross, M. Sedegah, M. Leef, T. Theisin, F.A. Robey, S.L. Hoffman and R.L. Beaudoin. Naval Medical Research Institute, Bethesda, MD; Smith Kline & French Laboratory, Swedeland, PA; PAHO, Washington, DC; National Institute of Dental Research, Bethesda, MD.
- 11:45 385 HELPER T CELLS ARE REQUIRED DURING THE INDUCTION OF CELL-MEDIATED IMMUNITY TO MALARIA SPOROZOITES. W.R. Weiss, M. Sedegah and R.L. Beaudoin. Naval Medical Research Institute, Bethesda, MD.

THURSDAY MORNING - DECEMBER 8

SCIENTIFIC SESSION T: AMEBIASIS AND GIARDIASIS

8:45 AM - 12:00 Noon

Executive

Chairpersons: W.A. Petri, Jr. and S.L. Reed

Time    Abst

- 8:45 386 CYSTEINE PROTEINASE EXPRESSION AND PATHOGENICITY OF ENTAMOEBA HISTOLYTICA. S.L. Reed,\* W.E. Keene and J.H. McKerrow. University of California at San Diego, CA; University of California at San Diego Medical Center, San Francisco, CA.
- 9:00 387 MUCUS SECRETAGOGUE ACTIVITY OF ENTAMOEBA HISTOLYTICA IN RAT COLONIC LOOPS. K. Chadee,\* D.J. Innes and J.I. Ravdin. Institute of Parasitology, McGill University, Montreal, CANADA; University of Virginia School of Medicine, Charlottesville, VA.
- 9:15 388 BINDING OF THE ENTAMOEBA HISTOLYTICA GAL/GALNAC INHIBITABLE ADHERENCE LECTIN TO GLYCOSYLATION DEFICIENT CHINESE HAMSTER OVARY CELL MUTANTS. J.I. Ravdin,\* P. Stanley, C.F. Murphy and W.A. Petri, Jr. University of Virginia School of Medicine, Charlottesville, VA; Albert Einstein College of Medicine, Bronx, NY.

- 9:30 389 CHINESE HAMSTER OVARY CELLS DEFICIENT IN N-ACETYLGUCOSAMINYL-TRANSFERASE I ARE RESISTANT TO E. HISTOLYTICA-MEDIATED CYTOTOXICITY. E. Li,\* A. Becker and S.L. Stanley, Jr. Washington University School of Medicine, St. Louis, MO.
- 9:45 390 OLIGONUCLEOTIDE PROBES TO THE CELL SURFACE ADHERENCE LECTIN OF ENTAMOEBIA HISTOLYTICA. B.J. Mann,\* T. Snodgrass, E.L.W. Kittler, J.I. Ravdin and W.A. Petri, Jr. Departments of Medicine and Microbiology, University of Virginia, Charlottesville, VA.
- 10:00 COFFEE BREAK
- 10:30 391 HYBRID FORMATION IN ENTAMOEBIA HISTOLYTICA. E. Orozco,\* F. Solis and M.A. Vargas. Department of Genetics. CINVESTAV I.P.N. MEXICO D.F.
- 10:45 392 DETECTION OF E. HISTOLYTICA IN STOOL SAMPLES BY DNA SPOT HYBRIDIZATION. R. Acuna-Soto, J.C. Samuelson, F. Biagi and DF Wirth. Department of Tropical Public Health, Harvard School of Public Health and Department of Pathology, Brigham and Women's Hospital, Boston, MA.
- 11:00 393 MURINE T-CELL CLONES AGAINST ENTAMOEBIA HISTOLYTICA: IN VIVO AND IN VITRO CHARACTERIZATION. M. Denis\* and K. Chadee. Institute of Parasitology of McGill University, Ste-Anne-de-Bellevue, Quebec, CANADA.
- 11:15 394 DEVELOPMENT OF CELL MEDIATED IMMUNITY IN PEYER'S PATCHES OF GIARDIA MURIS-INFECTED MICE. D.R. Hill and R. Pohl.\* Division of Infectious Diseases, University of Connecticut School of Medicine, Farmington, CT.
- 11:30 395 ANTIGENIC VARIATION OF GIARDIA LAMBLIA IN HUMAN EXPERIMENTAL INFECTIONS. T.E. Nash, D.A. Harrington, G.A. Losonsky, M.M. Levine, J.T. Conrad and J.W. Merritt, Jr. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD; Center for Vaccine Development, University of Maryland, Baltimore, MD.
- 11:45 396 ENCYSTATION-SPECIFIC ANTIGENS OF GIARDIA LAMBLIA. D.S. Reiner,\* H. Douglas and F.D. Gillin. University of California at San Diego Medical Center, San Diego, CA.

THURSDAY MORNING - DECEMBER 8

SCIENTIFIC SESSION U: FILARIASIS - SURFACE ANTIGENS

8:15 AM - 12:00 Noon

Congressional

Chairpersons: A.L. Scott and G.J. Weil

<u>Time</u>	<u>Abst</u>	
8:15	397	ORGANIZATION, SYNTHESIS AND STRUCTURE OF CUTICULAR PROTEINS OF <u>BRUGIA SPP.</u> M.E. Selkirk,* M. Yazdanbakhsh, M. Blaxter, W. Gregory, G.E. Kwan-Lim, E. Cookson, W. Paxton and R.M. Maizels. Imperial College of Science and Technology, London, ENGLAND.
8:45	398	BIOCHEMICAL AND IMMUNOLOGICAL CHARACTERIZATION OF THE SURFACE ANTIGENS FROM ADULT <u>DIROFILARIA IMMITIS.</u> A.L. Scott, D.A. Moraga, M.S. Ibrahim and W.K. Tamashiro. Department of Immunology and Infectious Diseases, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD.
9:00	399	ANALYSIS AND AFFINITY PURIFICATION OF CUTICULAR PROTEINS OF <u>BRUGIA MALAYI</u> AFTER BIOTINYLATION OF INTACT ADULT PARASITES. R.M. Alvarez* and G.J. Weil. The Jewish Hospital at Washington University Medical Center, St. Louis, MO.
9:15	400	QUALITATIVE CHARACTERIZATION OF ANTIBODY RESPONSES OF JIRDS TO <u>BRUGIA PAHANGI</u> INFECTION. R.G. Farrar,* T.R. Klei, C. McVay and S.U. Coleman. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.
9:30	401	MOLECULAR MIMICRY OF A SURFACE EPITOPE OF <u>BRUGIA MALAYI</u> INFECTIVE LARVAE BY ANTI-IDIOTYPIC ANTIBODIES. K.S. Carlow Clotilde,* P. Busto and M. Philipp. Molecular Parasitology Group, New England Biolabs, Beverly, MA.
9:45	402	ISOLATION AND PARTIAL CHARACTERIZATION OF RECOMBINANT ANTIGENS FROM A GENOMIC LIBRARY OF <u>WUCHERERIA BANCROFTI.</u> N. Raghavan,* C.V. Maina, L.A. McReynolds and T.B. Nutman. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; New England Biolabs, Beverly, MA.
10:00		COFFEE BREAK
10:30	403	ANTIGEN SHEDDING FROM THE SURFACE OF <u>DIROFILARIA IMMITIS</u> INFECTIVE LARVAE. M.S. Ibrahim, W.K. Tamashiro, D.A. Moraga and A.L. Scott. Department of Immunology and Infectious Diseases, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD.
10:45	404	PURIFICATION AND BIOCHEMICAL AND IMMUNOLOGIC CHARACTERIZATION OF A 25KD GLYCOPROTEIN FROM THE SURFACE OF <u>DIROFILARIA IMMITIS</u> FOURTH STAGE LARVAE. T.B. Davis* and M. Philipp. Molecular Parasitology Group, New England Biolabs, Beverly, MA.

- 11:00 405 A 16 KD SURFACE ANTIGEN OF BRUGIA MALAYI EXPRESSED BY FOURTH STAGE LARVAE, ADULT WORMS AND BY POST-PARASITIC THIRD STAGE LARVAE. N. Storey and M. Philipp. Molecular Parasitology Group, New England Biolabs, Beverly, MA.
- 11:15 406 A HYDROPHOBIC GLYCOSYLATED COMPONENT ON THE SURFACE OF ONCHOCERCA LIENALIS MICROFILARIAE. D.E. Hill,\* J.J. Donnelly, M. Khatami, J.B. Lok and J.H. Rockey. University of Pennsylvania, Philadelphia, PA.
- 11:30 407 DIFFERENTIAL RECOGNITION OF A MICROFILARIA-SPECIFIC ANTIGEN OF BRUGIA MALAYI. W.F. Piessens,\* L. Kurniawan and E. Basundari. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Badang Kesehatan, Jakarta, INDONESIA.
- 11:45 408 STUDIES ON ANTI-MICROFILARIAL IMMUNITY USING DIROFILARIA IMMITIS IN LEWIS RATS. W.K. Tamashiro,\* M.S. Ibrahim, D.A. Moraga and A.L. Scott. Departments of Immunology and Infectious Diseases, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD.

THURSDAY AFTERNOON - DECEMBER 8

WORKSHOP: CELL-MEDIATED IMMUNITY TO ASEXUAL MALARIA PARASITES

1:30 PM - 5:00 PM

Ambassador

Chairperson: L.H. Miller

Time    Abst

- 409 INTRODUCTION. L.H. Miller. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD; W.P. Weidanz, Hahnemann University School of Medicine, Philadelphia, PA; J.H.L. Playfair, Middlesex Hospital Medical School, London, ENGLAND; and I.A. Clark, Australian National University, Canberra, AUSTRALIA.
- 410 K.N. Brown. National Institute of Medical Research, Mill Hill, UNITED KINGDOM.
- 411 S. Kumar. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD.
- 412 R. Mogil. University of Alberta, Edmonton, Alberta, CANADA.
- 413 D.J. Wyler. Tufts University School of Medicine, Boston, MA.

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\* This Workshop is supported by the Agency for International Development Malaria Immunity and Vaccine Research Program.

THURSDAY AFTERNOON, DECEMBER 8

ANNUAL SCIENTIFIC MEETING OF THE AMERICAN COMMITTEE OF MEDICAL ENTOMOLOGY (ACME)

RECENT APPROACHES TO THE STUDY OF SYSTEMATICS AND EVOLUTION OF  
ARTHROPOD VECTORS AND ARTHROPOD-TRANSMITTED PATHOGENS

1:15 PM - 5:00 PM

Diplomat

Chairpersons: B.F. Eldridge

<u>Time</u>	<u>Abst</u>	
1:15	414	INTRODUCTION AND SCOPE. B.F. Eldridge. Department of Entomology, University of California, Davis, CA.
1:30	415	CURRENT TRENDS IN STUDIES OF THE GENETIC STRUCTURE OF VECTOR POPULATIONS. L.E. Munstermann. Vector Biology Laboratory, University of Notre Dame, IN.
2:00	416	RECENT DEVELOPMENTS IN GENETICS AND SYSTEMATICS OF TICKS. J.H. Oliver, Jr. Department of Biology, Georgia Southern College, Statesboro, GA.
2:30	417	THE USE OF RIBOSOMAL AND MITOCHONDRIAL DNA TO STUDY POPULATION AND SPECIES RELATIONSHIPS IN ANOPHELINES. F.H. Collins. Centers for Disease Control, Atlanta, GA.
3:00		COFFEE BREAK
3:15	418	A GENETIC APPROACH TO THE STUDY OF TICK TRANSMITTED ARBOVIRUSES (ORBIVIRUSES). P.A. Nuttall. National Environment Research Council, Institute of Virology, Oxford, UNITED KINGDOM.
3:45	419	IDENTIFICATION OF SPECIES OF <u>PLASMODIUM</u> . R.A. Wirtz. Department of Entomology, Walter Reed Army Institute of Research, Washington, DC.
4:15	420	MOLECULAR IDENTIFICATION AND CLASSIFICATION OF SPECIES OF <u>LEISHMANIA</u> . D. McMahon-Pratt. Yale University School of Medicine, New Haven, CT.
4:45		DISCUSSION AND QUESTIONS.

THURSDAY AFTERNOON - DECEMBER 8

SCIENTIFIC SESSION V: RETROVIRAL INFECTIONS AND EPIDEMIOLOGY

1:30 PM - 5:00 PM

Palladian

Chairpersons: N. Constantine and H. Spencer

<u>Time</u>	<u>Abst</u>	
1:30	421	HUMAN EOSINOPHILS CAN EXPRESS CD4 AND HLA-DR, AND BIND HIV GP120. D.R. Lucey,* A. Nicholson-Weller and P.F. Weller. Harvard Medical School, Beth Israel Hospital, Boston, MA.
1:45	422	HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION IN EGYPTIANS IN CAIRO. F.S. Galal,* M. Kamal, M. Haphez, Y. Safwat, N. Bassiouni, Z. Farid and J.N. Woody. Abbassia Fever Hospital (AFH), United States Naval Medical Research Unit No. 3 and the Ministry of Health, Cairo, EGYPT.
2:00	423	SEROLOGIC EVIDENCE FOR HIV-2 IN EAST AFRICA. N.T. Constantine,* D.M. Watts, R.L. Daise and J.N. Woody. United States Naval Medical Research Unit No. 3, Cairo, EGYPT.
2:15	424	HUMAN IMMUNODEFICIENCY VIRUS IN SUDANESE PROSTITUTES. M.C. McCarthy,* N.T. Constantine, R.L. Daise and E. Magoub. United States Naval Medical Research Unit No. 3, Cairo, EGYPT; Ministry of Health, Khartoum, SUDAN.
2:30	425	HIGH PREVALENCE RATES OF HUMAN T-LYMPHOTROPIC VIRUS TYPE I (HTLV-I) INFECTION IN ISOLATED POPULATIONS OF THE WESTERN PACIFIC WITHOUT JAPANESE OR AFRICAN CONTACT. R.M. Garruto,* P. Slover, C. Mora, R. Yanagihara, D.M. Asher, P. Rodgers-Johnson and D.C. Gajdusek. National Institutes of Health, Bethesda, MD.
2:45	426	THE PREVALENCE OF HUMAN IMMUNODEFICIENCY VIRUS AND HEPATITIS B VIRUS INFECTION IN HIGH RISK GROUPS IN CAIRO. S. Bassily,* N.T. Constantine, J.N. Woody and F. Sheiba. United States Naval Medical Research Unit No. 3 and Ministry of Health, Cairo, EGYPT.
3:00		COFFEE BREAK
3:30	427	EPIDEMIOLOGY OF HTLV-I INFECTION IN REMOTE COASTAL POPULATIONS ON THE PHILIPPINE ISLAND OF PALAWAN. C.G. Hayes,* C.R. Manaloto, R.B. Oberst and L.W. Laughlin. United States Naval Medical Research Unit No. 2, Manila, THE PHILIPPINES.
3:45	428	NEUROBEHAVIORAL EFFECTS OF TOXOCARIASIS. T.M. Bailey,* P. Succop, P.M. Schantz, R.L. Bornschein, K.N. Dietrich, J.K. Stehr-Green. Centers for Disease Control, Atlanta, GA; Cincinnati Lead Study, University of Cincinnati Medical Center, Cincinnati, OH.

- 4:00 429 GUINEA WORM PILOT CONTROL PROJECTS IN TWO PAKISTANI VILLAGES. R. Imtiaz, T.M. Bailey, J.D. Anderson, M. Burney, E. Ruiz and B.L. Cline. Global 2000, Inc. and Centers for Disease Control, Atlanta, GA; National Institutes of Health, Islamabad, PAKISTAN.
- 4:15 430 PAKISTAN: NATIONWIDE SEARCH FOR GUINEA WORM DISEASE (GWD). M.A. Rab, R. Imtiaz, J.D. Anderson, E. Shafa, A. Munir, E. Ruiz, M. Burney and B.L. Cline. National Institutes of Health, Islamabad, PAKISTAN; Global 2000, Inc.; and Centers for Disease Control, Atlanta, GA.
- 4:30 431 DRACUNCULIASIS ERADICATION IN NIGERIA: PROSPECTS AND CHALLENGES. L.D. Edungbola\*. Faculty of Health Sciences, University of Ilorin, NIGERIA.
- 4:45 432 ASSOCIATION OF HEPATITIS E VIRUS (HEV) WITH AN EPIDEMIC OF ENTERICALLY TRANSMITTED NON-A, NON-B HEPATITIS (ENANBH) IN PAKISTAN: DETECTION OF ANTI-HEV IN SERUM AND HEV IN FECES. J. Ticehurst,\* T.J. Popkin, J.P. Bryan, B.L. Innis, J.F. Duncan, A. Ahmed, A.Z. Kapikian, M. Iqbal, L.J. Legters and R.H. Purcell. Walter Reed Army Institute of Research, Washington, DC; NIAID and USUHS, Bethesda, MD; Armed Forces Research Institute Medical Sciences, Bangkok, THAILAND; Pakistan-United States Laboratory of Seroepidemiology, Army Medical College, Rawalpindi, PAKISTAN.

THURSDAY AFTERNOON - DECEMBER 8

SCIENTIFIC SESSION W: SCHISTOSOMIASIS - IMMUNOLOGY

1:30 PM - 5:00 PM

Executive

Chairpersons: D. Harn and S. James

Time Abst

- 1:30 433 IgE MONOCLONAL ANTIBODY TO SCHISTOSOMA MANSONI: SPECIFICITY, PARTIAL PURIFICATION OF ANTIGEN, AND APPLICATION FOR ANTIGEN DETECTION. F.N. Boctor,\* J. Smith, K.A. Shelby, K. Kamal, A. Al Gauhari and J.B. Peter. United States Naval Medical Research Unit No. 3, Cairo, EGYPT; Specialty Laboratories, Inc., Santa Monica, CA.
- 1:45 434 ROLE OF CLONED T LYMPHOCYTE SUBSETS IN SCHISTOSOMULE KILLING. S.R. Reynolds\* and G.I. Higashi. Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI.
- 2:00 435 THE REGULATION OF IMMUNITY IN SCHISTOSOMIASIS BY AN IDIOTYPICALLY AND GENETICALLY RESTRICTED T-CELL DERIVED SUPPRESSOR-EFFECTOR FACTOR. P.J. Perrin,\* L. Jiaoqiao and S.M. Phillips. University of Pennsylvania School of Medicine, Philadelphia, PA.

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- 2:15 436 PARTIAL PURIFICATION AND IDENTIFICATION OF EOSINOPHIL STIMULATION PROMOTER: A COMBINATION OF GM-CSF AND IL-5. W.E. Secor,\* S.J. Stewart and D.G. Colley. Vanderbilt University School of Medicine and Veterans Administration Medical Center, Nashville, TN.
- 2:30 437 A SERUM FACTOR SUPPRESSES TNF-MEDIATED ACTIVATION OF HUMAN EOSINOPHIL TOXICITY TO SCHISTOSOMULA OF S. MANSONI. D.S. Silberstein,\* M.S. Minkoff and J.R. David. Harvard Medical School and Brigham and Women's Hospital, Boston, MA.
- 2:45 438 FRACTIONATED SERA FROM SCHISTOSOMA MANSONI INFECTED PATIENTS CONFERS PASSIVE PROTECTION IN MICE. J. Jwo and P.T. LoVerde.\* Department of Microbiology, State University of New York, Buffalo, NY.
- 3:00 COFFEE BREAK
- 3:30 439 LYMPHOID PHENOTYPIC ALTERATIONS IN MURINE SCHISTOSOMIASIS MANSONI; WITH AND WITHOUT SUPPRESSOR FACTOR ADMINISTRATION. G.S. Henderson,\* T.L. McCurley and D.G. Colley. Vanderbilt University School of Medicine and V.A. Medical Center, Nashville, TN.
- 3:45 440 MECHANISM OF SCHISTOSOMULUM KILLING BY LYMPHOKINE-ACTIVATED MACROPHAGES. S.L. James and J. Glaven. George Washington University Medical Center, Washington, DC.
- 4:00 441 TARGET ANTIGENS OF HOST ANTIBODIES INVOLVED IN THE CHEMOTHERAPY OF SCHISTOSOMA MANSONI WITH PRAZIQUANTEL (PZQ). P. Brindley,\* M. Strand and A. Sher. LPD, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD; Johns Hopkins University, Baltimore, MD.
- 4:15 442 FOUR MAJOR SURFACE MOLECULES OF SCHISTOSOMA MANSONI ARE ANCHORED TO THE SCHISTOSOMULUM MEMBRANE BY GLYCOSYLPHOSPHATIDYLINOSITOL (GPI). D.J. Pearce\* and A. Sher. ICBS, LPD, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD.
- 4:30 443 MODULATION OF EGG GRANULOMAS IN MICE CHRONICALLY INFECTED WITH SCHISTOSOMA MANSONI IS ASSOCIATED WITH DECREASED PRODUCTION OF FIBROBLAST STIMULATING FACTORS. S. Prakash,\* D.J. Wyler and A.E. Postlethwait. New England Medical Center Hospitals, and Tufts University School of Medicine, Boston, MA; University of Tennessee, Memphis, TN.
- 4:45 444 LYMPHOKINE MESSENGER RNA LEVELS IN SPLEEN TISSUE FROM PATIENTS WITH HEPATOSPLENIC SCHISTOSOMIASIS. M.D. Ricciardon,\* K.A. Kamal, M.M. Mansour and J.N. Woody. United States Naval Medical Research Unit No. 3, Cairo, EGYPT.

## PLENARY SESSION

WELCOME. J.K. Frenkel. President, ASTMH.

- 1 ANTIGEN PRESENTATION. Antonio Lanzavecchia. Basel Institute for Immunology, Basel, SWITZERLAND.
- 2 T CELL RECOGNITION: APPLICATIONS TO MALARIA AND AIDS. Jay A. Berzofsky. National Cancer Institute, National Institutes of Health, Bethesda, MD.
- 3 REGULATION OF IMMUNOGLOBULIN EXPRESSION BY LYMPHOKINES. William E. Paul. Chief, Laboratory of Immunology, National Institutes of Health, Bethesda, MD.
- 4 THE ACTIVATED MACROPHAGE AND PROTOZOAL INFECTION. Henry W. Murray. Professor of Medicine and Head, Division of Infectious Diseases, Cornell University Medical College, New York, NY.

## CLINICAL TROPICAL MEDICINE GROUP MEETING

- 5 CPC: INCONTINENCE AND RIGHT LEG PARESIS IN A 37 YEAR OLD MAN FROM GUATEMALA. DISCUSSANT: M. Barry. Yale University School of Medicine, New Haven, CT. PATHOLOGIST: M. Wittner. Albert Einstein College of Medicine, New York, NY.
- 6 UPDATE ON THE DIAGNOSIS AND TREATMENT OF LARVAL TAPEWORM DISEASE. P.M. Schantz. Centers for Disease Control, Atlanta, GA.
- 7 DIAGNOSTIC DILEMMAS: PROVOCATIVE CASES IN TROPICAL MEDICINE. J.S. Keystone, University of Toronto, Ontario, CANADA.
- 8 UPDATE ON ANTIMALARIAL CHEMOPROPHYLAXIS: DOXYCYCLINE, PROGUANIL AND FANSIDAR. C.C. Campbell and H.O. LobeI. Malaria Branch, Centers for Disease Control, Atlanta, GA.

### BUSINESS MEETING.

Chairperson: M.S. Wolfe. Department of State, Washington, D.C.

## CLINICO-PATHOLOGIC CONFERENCE

### INCONTINENCE AND RIGHT LEG PARESIS IN A 37 YEAR OLD MAN FROM GUATEMALA

A 37 yo Guatemalan male was well until 1/23/88 when he had the onset of intermittent upper back pain radiating toward the shoulders. He was able to work until 3/2/88 when he first noted the inability to void urine. Two weeks later urinary incontinence and constipation occurred. At this time he began to have daily spiking chills and fever to 39°C, night sweats, myalgias, and a productive cough. He was treated with Ampicillin and Tylenol with no change in these symptoms.

Two weeks before admission he had the onset of progressive left extremity weakness to the point of being unable to rise from a supine position. He also was unable to have penile erections and he noted a decrease in perianal sensation. He persisted in having night sweats as well.

The patient had come to the U.S. 2 years prior to admission and had not been back to Guatemala. He worked as a masonry foreman, was married and lived with his wife and 4 children. He denied having any homosexual or extramarital affairs. The patient had used heroin more than 10 years prior to admission, for several months. He has not used intravenous drugs since that time. All his family members are well except for his younger sister, who lives in Guatemala. She developed late onset seizures 3 years ago. The patient was allergic to shellfish. He was admitted for myelography.

On physical examination the patient was a well developed anxious male in no acute distress. He was diaphoretic with a rectal temperature of 38.5°C. The rest of the physical examination was unremarkable except for the neurological examination. The left pupil was 4 mm in diameter and reacted to light and accommodation. He had been blind in the right eye since birth. The motor examination of the upper extremities was normal except for distal intrinsic weakness bilaterally. Most of the significant findings were limited to the motor function of the lower extremities: Iliopsoas L 2+, R 0+; gluteus R 4+, L 2+; hamstrings L 4+, R 0+; quadriceps L 2+, R 0+; tibialis anterior L 3+, R 0+; gastrocnemius L 3+, R 0+. Perianal sensation was markedly diminished. He was incontinent of urine and feces; he was unable to have an erection.

The myelogram revealed a block at T3. X-rays of the spine were considered normal. A CT scan of the spine revealed swelling at T3-T4. Soft tissue x-rays were unrevealing. Laboratory data: Na 146 mg/dl; Cl 106 mg/dl; K 4.4 mg/dl; CO<sub>2</sub> 26 meq/L; BUN 34 mg/dl; creatinine 1.1 mg/dl; glucose 131 mg/dl; Ca 10.3 mg/dl; P 6.0 mg/dl; albumin 4.4 g/dl; uric acid 3.3 mg/dl. Liver function tests: alkaline phosphatase 140 (nl 80); LDH 259; SGOT/PT 15/14. Hematocrit 39%; WBC 6200/mm<sup>3</sup> (57P, 15L, 8M); platelets 336000/mm<sup>3</sup>. Stool examination revealed cysts of Giardia, Blastocystis hominis. PPD was negative; skin tests for Candida and mumps were positive. HIV-1 serology was negative.

A surgical procedure was performed on the fourth hospital day.

SYMPOSIUM: MALARIAL IMMUNITY IN MICE AND HUMANS

9-15 An update of mechanisms that lead to protective immunity and their implications in antimalarial vaccination will be presented. The symposium should be of interest to individuals working in malarial immunity as well as those studying immune responses to other infectious organisms. The opening presentation by D.W. Taylor will compare humoral responses to erythrocyte-, gametocyte- and sporozoite-stage parasites. Emphasis will be on mechanisms of antibody production and regulation including T helper cells, B cell subsets, isotype-expression, polyclonal activation and antibody catabolism. The following two presentations will consider the role of cell-mediated immunity (CMI) in resistance to malaria. Melacon-Kaplan and Weidanz will provide evidence for a role for CMI during acute blood-stage infections. They will also present evidence for possible mechanisms of T dependent immunity including the activation of effector cells (NK cells, PMN and macrophages) and the role of the spleen. J. Playfair will discuss the role of CMI mediated by T cells and other effector cells including macrophages, neutrophils, eosinophils, endothelial cells, and NK cells with special emphasis on enhancement of the mechanism by vaccination. Oxidative and non-oxidative mechanisms of parasite killing and the role of the liver will be presented. The role of macrophages during malaria infection will be presented by H.L. Shear. In particular, the activation of these cells by T cell-derived lymphokines will be considered as well as the implications of macrophage activation for antimalarial vaccination and prophylaxis. A discussion of additional non-antibody mediated mechanisms of parasite destruction, in particular, human crisis forming factor (CFF), will be delivered by J.B. Jensen. CFF is a unique factor present in the sera of immune individuals that can be distinguished from tumor necrosis factor (TNF) and reactive oxygen species. I.A. Clark will describe the production of mediators of inflammation with emphasis on TNF. The influence of TNF on malarial immunity and pathogenesis will be considered, as well as the interaction of TNF and other inflammatory mediators including lymphokines, IL-1, prostoglandins, reactive oxygen species and platelet-activation factor. The symposium will conclude with M.M. Stevenson providing evidence for the genetic control of innate and acquired immunity to the exoerythrocytic and erythrocytic stages of malaria in mice and man. She will also discuss genetic control of immune responses to P. falciparum sporozoite vaccines.

A: ARBOVIROLOGY - ARBOVIRAL ENTOMOLOGY

TRANSSTADIAL AND HORIZONTAL TRANSMISSION OF RIFT VALLEY FEVER VIRUS IN  
THE TICK HYALOMMA TRUNCATUM

- 16 K.J. Linthicum,\* T.M. Logan, C.L. Bailey, D.J. Dohm, J.R. Moulton. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21701.

To assess the role that ticks might play as enzootic/epizootic vectors of Rift Valley fever virus (RVFV), laboratory experiments were designed to follow virus replication and transmission in Hyalomma truncatum and Rhipicephalus appendiculatus. Ticks were exposed to RVFV by intracoelomic inoculation or by feeding on a viremic hamster and assayed for virus on Vero cell monolayers at predetermined intervals. The virus replicated in H. truncatum after intracoelomic inoculation. Viral titers peaked in fed male ticks after dropping off a host [mean titer =  $10^{4.3}$  plaque forming units (PFU)]. Adult inoculated H. truncatum transmitted virus to hamsters. Virus persisted at least 58 days in these ticks. Virus was also shown to pass transstadially from inoculated H. truncatum nymphs to adults, with peak viral titers reaching  $10^{3.5}$  PFU in adult males after feeding. Virus was recovered from females 121 days after inoculation of nymphs. Hyalomma truncatum larvae and nymphs, given a RVFV infected bloodmeal did not become infected. In R. appendiculatus adults, viral titers declined rapidly and were undetectable by 12 days post inoculation. As differences exist between tick species with regard to infection with RVFV, other potential vector species should be investigated. Although previous studies failed to demonstrate RVFV replication in ticks, our study indicates that a persistent RVFV infection in ticks parasitizing animals that migrate from areas of enzootic/epizootic disease could explain how the virus was introduced into Egypt in 1977 and into the Senegal River basin in 1987.

CRIMEAN-CONGO HEMORRHAGIC FEVER IN SENEGAL: INFECTION RATES AND  
EPIDEMIOLOGIC ASSOCIATIONS OF TICK VECTORS AND VERTEBRATE HOSTS.

- 17 M. L. Wilson\*, B. LeGuanno, J.-L. Camicas, J.-P. Cornet, J.-F. Saluzzo, J.-P. Gonzalez and J.-P. Digoutte. Institut Pasteur, B.P.220, Dakar, Senegal; Dept. of Tropical Public Health, Harvard University, Boston, MA; Laboratoire ORSTOM de Zoologie medicale de l'Institut Pasteur; Disease Assessment Division, USAMRIID, Fort Detrick, Fredrick, MD.

Transmission of Crimean-Congo Hemorrhagic Fever virus, family Bunyviridae, is focally enzootic throughout Africa and southern Eurasia. Zoonotic disease sporadically erupts in localized epidemics causing significant morbidity and mortality. The ecological factors that permit "silent" maintenance or reintroduction of the virus, and that promote epizootics or epidemics, remain obscure. In sub-saharan West Africa, our research is directed toward analysis of tick-host population dynamics, quantification of horizontal and vertical transmission rates, identification of candidate reservoirs, and evaluation of risk to human health. Initial results demonstrate that potential vector ticks, notably Hyalomma spp., are abundant and widespread; domestic ungulates host numerous adult ticks throughout the year, yet immature ticks, enigmatically rare, apparently feed primarily on birds and small mammals from August to November. Observations on vertebrate hosts suggest that certain species are important to vector reproduction, but not to virus transmission. Using suckling mouse inoculation and antigen-capture ELISA, adult ticks, eggs and unfed immatures are being tested to compare horizontal and vertical infection rates. Despite the extensive distribution of potential tick vectors, prevalence of infection varies considerably. Maternal transmission of IgG to offspring occurs. Incidence rates suggest that low-level transmission may be seasonal.

A: ARBOVIROLOGY - ARBOVIRAL ENTOMOLOGY

18 TISSUE TROPISMS AND REPLICATION STRATEGY OF DUGBE VIRUS (NAIROVIRUS, BUNYAVIRIDAE) IN AMBLYOMMA VARIEGATUM TICKS.

G.M. Steele, and P.A. Nuttall.\* NERC Institute of Virology, Mansfield Road, Oxford, OX1 3SR, U.K.

We have previously shown that the African tick species, Amblyomma variegatum, can act as a competent vector of Dugbe (DUG) virus, a Nairovirus in the Bunyaviridae. Experimental studies were undertaken to determine the course of viral infection through the various tissues and organs of the tick. Questing and feeding female ticks that had been infected as nymphs by either direct inoculation or capillary feeding, were dissected. Their tissues and organs were examined for DUG virus by trituration of the samples and inoculation of the clarified supernatant intracerebrally into 2 day-old mice, and PS and XTC cell cultures, and by indirect immunofluorescence of tick sections, squashes, or smears. During the questing stage, and 3 days post-attachment on uninfected rabbits or guinea pigs, DUG virus was only found in the gut and hemolymph. However, at 10 days post-attachment, DUG virus was detected in the gut, hemolymph, ovary, muscle, synganglion, and salivary gland, but not in the malpighian tubules. The highest titer of virus was consistently found in the gut. Timing of the appearance of DUG virus in the salivary fluids and gut contents were also investigated. The results indicate that the tick gut, besides being important in determining vector competence, appears to play a vital role in maintaining DUG virus in infected ticks and, possibly, in the transmission of the virus.

19 ANATOMICAL BASIS OF THOGOTO VIRAL INTERFERENCE IN THE TICK VECTOR, RHIPICEPHALUS APPENDICULATUS.

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Thogoto (THO) virus is a tick-borne arbovirus and a candidate member of the family Orthomyxoviridae. In previous studies employing the vectorally competent 3-host tick, R. appendiculatus, we have demonstrated that interference occurs following homologous superinfection of ticks with THO virus. Experimental studies were undertaken to investigate the role of the gut in viral interference. This was examined by:-

(i) inter-stadial infection:- ticks were orally infected at the larval stage with a temperature-sensitive (ts) mutant of THO virus by feeding on a viraemic hamster; following moulting the nymphs were fed on uninfected guinea pigs. Within a day of completing engorgement the nymphs were inoculated inter-coelomically with wild-type(wt) THO virus.

(ii) intra-stadial infection:- nymphs were orally infected with a ts mutant of THO virus by feeding on a viraemic hamster; following engorgement the nymphs were inoculated inter-coelomically with wt THO virus.

When nymphs were assayed for virus twelve days post-engorgement, only wt virus was detected; control nymphs infected with ts or wt virus remained infected with the same virus. These results indicate that, if the gut is bypassed, the superinfecting virus can successfully replicate in the tick. Thus, THO viral interference in ticks appears to occur at the level of the gut.

A: ARBOVIROLOGY - ARBOVIRAL ENTOMOLOGY

- 20 TRANSOVARIAN TRANSMISSION OF AFRICAN SWINE FEVER VIRUS BY  
ORNITHODOROS MOUBATA.  
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N.Y.

The observation that transovarian transmission (TOT) of African swine fever virus (ASFV) occurs in its natural vector, Ornithodoros moubata, was based on data from 3 gravid, infected female ticks collected in Kenya. Experimental studies on TOT of ASFV in O. moubata were undertaken because the results from studies on TOT of ASFV in other soft tick species were inconsistent with those reported for O. moubata. Ticks were infected by feeding on a viremic pig either as adults or as N1 which were then reared to adults. Of 134 virgin females fed on a viremic pig the percent mortality following gonotrophic cycles 1-5 was 31.3, 3.7, 20.1, 6.0 and 1.5% respectively. In contrast, the percent mortality for control females fed on normal pigs was 0.0, 0.0, 12.0, 56.0 and 4.0% respectively for gonotrophic cycles 1-6. The percent of females that produced infected progeny in each gonotrophic cycle was 3.7, 37.8, 77.6, 65.0 and 57.1% for cycles 1-5 respectively. The mean filial infection rate gradually increased in subsequent gonotrophic cycles. Of 27 females alive after 5 gonotrophic cycles, 6 (18.5%) survived ASFV infection and never produced infected progeny. Ticks that were infected with ASFV as N1 showed a different pattern of TOT. The contrast in mortality rate, TOT rate and filial infection rate between O. moubata adults infected either as adults or as N1 indicates that ASFV exerts a significant, lethal selection pressure on O. moubata populations.

- 21 DEVELOPMENT OF CONGENIC MOSQUITO LINES FOR THE STUDY OF MODULATION OF  
WESTERN EQUINE ENCEPHALITIS VIRUS  
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Berkeley, CA

Two distinct populations of Culex tarsalis become evident following peroral infection with WEE virus: those with high viral titers that can transmit virus per os and those with low viral titers that cannot transmit virus per os. This phenomenon of WEE viral modulation can also be demonstrated in female and male Cx. tarsalis following intrathoracic (IT) inoculation with  $10^{2.0}$  PFU/mosquito and extrinsic incubation for 3 days at 32°C.

Two lines of Cx. tarsalis have been genetically selected that allow virus to multiply to mean titers of  $10^{7.5}$  PFU/mosquito (i.e. high viral producer or HVP) or  $<10^{1.0}$  PFU/mosquito (i.e. low viral producer or LVP) after IT inoculation. The trait for HVP is incompletely dominant and appears to be controlled by a single autosomal gene. However, the HVP and LVP lines were derived from different parental strains of Cx. tarsalis which complicates attempts to identify the products of HVP and LVP genes. Therefore, a serial backcross mating scheme was adopted that produced an HVP line that is congenic to LVP except for the alleles controlling viral production. Studies are currently in progress to verify the mode of inheritance of the HVP/LVP traits and to characterize viral multiplication in these recently selected congenic HVP and LVP lines.

A: ARBOVIROLOGY - ARBOVIRAL ENTOMOLOGY

- 22 INFLUENCE OF RIFT VALLEY FEVER VIRAL INFECTION ON THE DAILY SURVIVORSHIP OF Aedes mcintoshi AND Aedes fowleri MOSQUITOES. L.A. Patrican,\* T.M. Logan, and C.L. Bailey. USAMRIID, Fort Detrick, Frederick, MD 21701

In 11 trials we examined the effect of Rift Valley fever (RVF) viral infection on the survival of Aedes mcintoshi and Aedes fowleri either inoculated or orally exposed to RVF virus. Ae. mcintoshi is thought to be a vector and maintenance host of RVF virus in Kenya. Ae. fowleri, an experimentally competent vector, breeds in dambos associated with RVF. Daily survivor rates were compared between infected and uninfected females after 4, 7, 9, 12, 16, 20, and 24 days. No significant differences were detected between orally infected and uninfected Ae. mcintoshi until day 20 (21/36 and 91/122, respectively) or after day 24 (17/35 and 7/19, respectively). In 3 trials, no significant differences were found between inoculated infected (22/263) and uninfected (21/235) Ae. mcintoshi after day 24. However, in 2 trials, daily survivor rates were significantly lower for inoculated infected females by day 7 (53/83 vs 89/105) and day 9 (54/80 vs 83/100). For Ae. fowleri, daily survivor rates did not differ significantly until day 16 (2 trials) for orally infected (114/193) and uninfected (172/223) females or after 24 days (2 trials) for inoculated infected (157/238) and uninfected (158/229) females. Infection, dissemination and transmission rates were determined for each trial and are similar to rates observed in vector competence studies (Turell and Patrican). Neither RVF virus or viral antigen was detected in 867 Ae. mcintoshi (499 males:368 females) progeny, resulting from virus inoculated females, tested by plaque assay on Vero cells, passage in Toxorhynchites amboinensis and ELISA. Greater numbers of Ae. mcintoshi mosquitoes need to be tested in order to demonstrate experimental transovarial transmission of RVF virus. This study suggests that RVF virus has no adverse effect on the daily survivorship of Ae. mcintoshi or Ae. fowleri, at least throughout the first gonotrophic cycle.

- 23 EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE ABILITY OF MOSQUITOES TO TRANSMIT OCKELBO VIRUS.

J.O. Lundstrom\* and M.J. Turell. Statens Bakteriologiska Laboratorium, Stockholm, Sweden, and U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701-5011.

We examined the effect of environmental temperature on the ability of four mosquito species (Culex pipiens, Culex torrentium, Aedes aegypti, and Aedes taeniorhynchus) to transmit Ockelbo virus. Temperature regimens included: 10°C (mean low air temperature in the enzootic area in Sweden during the transmission period), 17°C (mean daily temperature), 24°C (mean high temperature), and a variable temperature incubator programmed to mimic continually the actual temperature in the enzootic area. Mosquitoes engorged on viremic 2-day-old chickens and were tested at predetermined intervals for transmission to susceptible chickens. Dissemination of virus from the midgut to the hemocoel was directly related to both the dose ingested and the incubation temperature in the Aedes species, with viral dissemination to the hemocoel significantly delayed in mosquitoes held at 10°C as compared to those maintained at the other temperatures. The effects of incubation temperature were similar, but less dramatic in the Culex species. All species transmitted Ockelbo virus, with transmission occurring as early as 7 days after the infectious blood meal, even in mosquitoes held at 10°C. Environmental conditions that most closely approximated the conditions found in Ockelbo enzootic areas were found to maximize the ability of mosquitoes to transmit this virus.

- 24 EFFECT OF THE DEVELOPMENTAL STAGE AT INFECTION ON THE ABILITY OF ANOPHELES ALBIMANUS TO TRANSMIT RIFT VALLEY FEVER VIRUS. M.J. Turell. USAMRIID, Fort Detrick, Frederick, MD.

The ability of Anopheles albimanus to transmit Rift Valley fever (RVF) virus was determined for specimens inoculated at selected times during the developmental stages. All 17 female An. albimanus, inoculated as larvae at least 24 h before pupation, transmitted virus by bite to hamsters. In contrast, only 1/62 (2%) of those inoculated as adults transmitted virus to hamsters. Transmission rates decreased as age at the time of inoculation increased (e.g., transmission rates for specimens inoculated < 20 h before pupation, < 4 h after pupation, or > 24 h after pupation were 4/5 [80%], 1/3 [33%], and 1/7 [14%], respectively). Viral titers recovered from specimens were similar for all groups tested, regardless of age at infection or of transmission status. Thus, differences in transmission rates may have been due to site-specific (i.e., salivary gland) replication, rather than a generalized increase in viral replication in mosquitoes inoculated at an earlier age. Perhaps inoculation of larvae allowed virus to enter cells in the primordial tissue that was destined to become adult salivary glands, which might otherwise become refractory to infection during metamorphosis to the adult stage. Mosquitoes were infected by inoculation in this study; however, as infection is present from the youngest stage onward in transovarially infected mosquitoes, a similar increase in vector competence may occur after transovarial, as compared to horizontal infection in mosquitoes.

- 25 HOST-ADAPTIVE MODIFICATION OF LA CROSSE VIRUS: PRELIMINARY STUDIES \*G.V. Ludwig, J.E. Osorio, B.M. Christensen, and T.M. Yuill. University of Wisconsin-Madison, Madison, WI.

Cyclic transmission between vertebrate and invertebrate hosts is a characteristic of all arboviruses. Maintenance of such transmission cycles requires that virus enter and replicate in functionally distinct, host specific cell types. In this study, infection of white-tailed deer and Mongolian gerbils by La Crosse virus (LACV) resulted in higher titered viremias of greater duration when virus was administered by infected adult female mosquitoes than when administered by parenteral inoculation. Similar experiments showed that LACV varied in its infectivity in vertebrate and mosquito hosts depending on the host cell type from which stock virus was prepared. LACV plaque experiments indicated that significant shifts in virus population structure, as determined by plaque size, occur after a single passage in either vertebrate or invertebrate cells. Results imply that passage of La Crosse virus in either a vertebrate or invertebrate host may result in the production of virion populations which are better capable of infecting cells of the next host in the transmission cycle.

A: ARBOVIROLOGY - ARBOVIRAL ENTOMOLOGY

ORBIVIRUSES REPLICATE IN CELLS FROM CULICOIDES VARIIPENNIS

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S.J. Wechsler\*, W.C. Wilson. U.S. Department of Agriculture, Agricultural Research Service, Arthropod-borne Animal Diseases Research Laboratory, Laramie, WY.

The orbiviruses, bluetongue (BT) and epizootic hemorrhagic disease (EHD) viruses, are transmitted in North America by the insect vector, the biting midge Culicoides variipennis (CV), to ruminant hosts. Studies on virus replication in the insect vector have been hampered by the lack of a cell line from C.V. We have successfully started a cell line (CuVa) from this insect. Both BT and EHD viruses replicate well in CuVa cells although cytopathic effect is subtle - vacuolation of the cytoplasm. The rate of viral replication in CuVa cells is influenced by the temperature at which the cells are being maintained; faster at 37 C than at 21 C. Peak titers also are influenced by temperature. Polyacrylamide gel electrophoresis (PAGE) revealed few differences in RNA from virus replicated in CuVa cells as compared to virus from vertebrate cells. We feel these CuVa cells may be useful in investigations of orbiviruses and of other Culicoides-transmitted viruses.

MOSQUITO ECOLOGY IN SUBURBAN COMMUNITIES IN THE GREATER LOS ANGELES AREA OF CALIFORNIA, USA. W.K. Reisen\* and R.P. Meyer, Sch. Publ. Hlth., Univ. Calif., Berkeley, CA.

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St. Louis encephalitis (SLE) recently has emerged as a public health concern in the greater Los Angeles area, with 25 human cases confirmed since 1983. Epidemiological studies indicated that some of these cases were contracted at suburban residences, a habitat where mosquito ecology is poorly understood. During the SLE summer transmission (Jul-Aug) and spring amplification (Mar-Apr) seasons, mosquitoes were sampled in 2 residential communities using 7 methods to determine species composition, relative abundance, resting sites, reproductive age and the relative prevalence and productivity of back yard and peripheral breeding sites. Culex quinquefasciatus was most abundant in both adult and larval collections, followed by Cx. peus, Cs. incidens and Cx. tarsalis. Cx. quinquefasciatus was readily collected by CO<sub>2</sub> and gravid traps when abundant, but was better sampled by gravid traps when abundance was low. Cx. peus and tarsalis were best sampled by CO<sub>2</sub> traps in tree canopy (5 m). The abundance of all species was clumped at those few homes (<10%) with breeding, and was independent of home owner opinion of recent mosquito bites. Mosquito abundance in a community with peripheral breeding was approximately 6X the abundance in a community with only backyard sources. These data indicate that the adequate control of peripheral breeding sources may be sufficient to maintain mosquito abundance below the level at which SLE transmission may occur.

B: FILARIASIS - EPIDEMIOLOGY AND IMMUNOLOGY

28 KNOWLEDGE, ATTITUDES, AND PERCEPTIONS (KAP) OF ONCHOCERCIASIS:  
A SURVEY AMONG RESIDENTS IN SOME ENDEMIC AREAS IN GUATEMALA.

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To interrupt transmission of onchocerciasis, ivermectin distribution must achieve maximum coverage of affected populations every 6-12 months for several years. Since community acceptance is essential for the success of such an endeavor, we performed a KAP survey of onchocerciasis among 144 households in five rural communities in an endemic area of Guatemala.

Given the country's long-standing nodulectomy program, it was not surprising that all persons interviewed knew the disease "filaria" as a skin nodule which, in some way, damaged the eyes. Relatively few (24%) knew that "filaria" was caused by a worm and acquired by the bite of an insect. Most persons felt that cure could only be achieved with surgery. Yet onchocerciasis was not perceived as a serious health problem, since few persons (12%) mentioned "filaria" in a "free list" of illnesses that occur in the community. Other attitudes were also identified which might be serious barriers to long-term acceptance of a national chemotherapy initiative. Community education and promotion activities must be developed to promote appropriate treatment-seeking behavior while addressing reservations about the control efforts.

29 COMPARATIVE DENSITIES OF WUCHERERIA BANCROFTI MICROFILARIA IN PAIRED  
SAMPLES OF CAPILLARY AND VENOUS BLOOD. M.L. Eberhard,\* J.M. Roberts,

P.J. Lammie, and R.C. Lowrie, Jr. Centers for Disease Control, Atlanta, GA., LSU Medical Center, New Orleans, LA., and Delta Regional Primate Research Center, Covington, LA.

Concurrent finger-prick and venous blood samples were obtained from 43 patients infected with Wuchereria bancrofti. Microfilariae were counted in blood smears and on nuclepore filters. Based on the numbers of microfilariae in 20-cmm finger-prick samples, an expected (theoretical) number of microfilariae in venous samples was calculated. This expected value was compared to the actual number of microfilariae present in venous blood. The actual number of microfilariae present ranged from 0.7 to 30 times (median 3) less than expected. The exponential function  $y=16.74e^{.17x}$ ,  $r^2=.69$ , where  $y$ =venous counts and  $x$ =capillary counts, was found to accurately reflect the relationship between venous and capillary counts. Each unit (microfilaria) change in capillary blood would result in an expected 19% unit increase/decrease in venous blood. These results support strongly the theory that microfilariae are unevenly distributed in the blood system. The epidemiological implication is that persons with low or ultralow microfilaremia levels in venous blood may have a much greater pool of microfilaria available in capillaries. We believe that the present study explains, at least partially, why mosquitoes feeding on these kinds of microfilaria carriers frequently have a greater uptake of microfilariae than expected.

## B: FILARIASIS - EPIDEMIOLOGY AND IMMUNOLOGY

- 30 IMMUNOLOGIC AND PARASITOLOGIC CHARACTERIZATION OF BANCROFTIAN FILARIASIS IN A HAITIAN PEDIATRIC POPULATION  
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LSU Medical Center of New Orleans, LA; Centers for Disease Control, Atlanta, GA; Tulane Univ., Delta Regional Primate Center, Covington, LA; and the ICIDR Program Tulane Univ., New Orleans, LA.

In order to examine the immunological consequence of exposure to filarial infection, cross-sectional serological studies were undertaken on more than 100 serum samples from pediatric patients (18 months - 15 years) living in Leogane, Haiti, an area endemic for bancroftian filariasis. Antibody and antigen levels were analyzed qualitatively and quantitatively using microassays developed to use sera (<50 $\mu$ l) collected by finger prick. Parasite antigen specific IgG and IgE levels were determined by ELISA and were standardized with respect to a high titered control sera derived from a microfilaremic subject and from one with tropical pulmonary eosinophilia respectively. Preliminary comparison of IgG and IgE levels between adults (residing in the same endemic area) and children demonstrates that children have higher IgG levels than adults (10,754  $\pm$  2,528 units/ml vs. 6,608  $\pm$  1,359 units/ml) although both groups have comparable and low levels of IgE (~ 100 units/ml). The prevalence of microfilaremia in these groups is not equivalent, however (41% adults vs. 23% children). The present data do suggest a relationship between parasitemia and IgG levels in that 80% of microfilaremic children had IgG levels lower than the mean. Immunoblot analyses in progress will allow characterization of the parasite antigens which elicit this immunoreactivity. Results of an antigen detection test using polyclonal rabbit anti-*B. pahangi* antibody in a sandwich ELISA indicate that a positive antigen test (absorbance > negative control + 3.S.D.) is predictive of the presence either of microfilaremia or symptomatic infection in adults. Of antigen positive adults 79% were either microfilaremic or symptomatic. In contrast, only 37% of children who were antigen positive were microfilaremic. In sum, preliminary analysis of these data support the concept that patterns of anti-filarial immunologic reactivity may differ between children and adults. (Supported by AI-16315 and AI-24459).

- 31 An in vitro technique to discriminate transmitting mosquitoes infected with *Wuchereria bancrofti*. A.N. Hassan, I.S. Abd El Azim, and A.M. Gad\*. Research and Training Center on Vectors of Diseases, Ain Shams University, Cairo, Egypt.

Demonstration of human filaria transmission by its vectors is usually impaired due to the lack of a laboratory recipient host. In vitro capillary feeding was used to determine both the proportion of infected mosquitoes able to transmit infective filarial larvae (L<sub>3</sub>) and the number of L<sub>3</sub> egested by each mosquito at one feeding. 14 days post-infection with *Wuchereria bancrofti* individual females of *Culex pipiens* were immobilized and allowed to feed by introducing the proboscis into a capillary tube filled with a sucrose solution. The number of expelled L<sub>3</sub> was determined in each tube, retained L<sub>3</sub> were determined through dissection. Of the 60 test mosquitoes, 93% contained a total of 368 L<sub>3</sub> (X = 6.6) but only 61% transmitted to the capillaries. Transmitting females harboured 76% of the total L<sub>3</sub> (range 1-50, X = 8) and released 54% of the worms to the capillaries (range 1.35, X = 4.5). One fourth of the non-transmitters had 1 to 7 active L<sub>3</sub> in their proboscis which failed to escape to the capillaries, suggesting that a proboscis barrier would prevent the release of the parasite. The capillary feeding technique may thus be useful to discriminate infected transmitting and non-transmitting mosquitoes. Moreover, the common dissection technique overestimated the calculated infective rate by 40%. This fact suggests that more than one-third of the L<sub>3</sub> positive *Culex* cannot be infective.

## B: FILARIASIS - EPIDEMIOLOGY AND IMMUNOLOGY

- 32 EFFECTIVENESS OF PIRIMIPHOS-METHYL RESIDUAL HOUSE SPRAYING FOR THE CONTROL OF MANSONIA BONNAE, THE VECTOR OF BRUGIAN FILARIASIS. M.S. Chang,\* N. Jute, and J. Lah. Vector Borne Diseases Control Programme, Medical Department, Sarawak, Malaysia.

Previous studies have shown that chemotherapy alone may not achieve interruption of transmission of Brugia malayi. A balanced, integrated control program employing both chemotherapy and vector control is under consideration by health authorities. Only limited laboratory trials and a single field evaluation of malathion fogging against Ma. bonnae and Ma. dives have been conducted. This study reports the results of a field trial with Pirimiphos-methyl. Pirimiphos-methyl 50 EC formulation was relatively simple to apply, with no indications of toxicity to the spraymen nor of any complaints from the local populace regarding odor or other objectionable characteristics. Applied at 2g ai/m<sup>2</sup>, it had a fairly long residual life, killing exposed mosquitoes (Ma. bonnae) at up to 50% of original application level for over 9 weeks. Natural mosquito population density was reduced by 75% indoors and 22% outdoors. As a result of the application, a marked decrease in the infective mosquito biting frequencies was observed. There were indications that Pirimiphos-methyl had a favorable impact on Brugian filariasis transmission in the treated village. The application of Pirimiphos-methyl appeared to be both efficacious and acceptable. Further studies on a larger scale for which careful epidemiologic surveillance is incorporated in the study to properly evaluate the merits of this promising insecticide formulation and the optimum dosage for Brugian filariasis vector control are needed.

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FIELD TEST OF DNA PROBES FOR BRUGIA MALAYI AND BRUGIA PAHANGI IN INDONESIA, S. Williams\*#, A. Salim<sup>+</sup>, C. Poole\*#, L. McReynolds#, Purnomo<sup>+</sup>, and F. Partono<sup>+</sup>. \*Dept. of Biological Sciences, Smith College, Northampton, Mass.; #New England Biolabs, Beverly, Mass. <sup>+</sup>Dept. of Parasitology, University of Indonesia, Jakarta, Indonesia.

We have developed species-specific DNA probes for both Brugia malayi and Brugia pahangi. These probes were used in a WHO sponsored double-blind study to determine if B. pahangi microfilariae (mf) can be found in humans from South Kalimantan, Indonesia. The study also included mf from laboratory infected cats and cats from the endemic area. The species and number of mf in each sample was determined both by the DNA probes and by morphology. Using the DNA probes, 26 laboratory cats were found to be infected with B. malayi, and ten were found to be infected with B. pahangi. Identical results were obtained by morphological examination of the blood samples. By morphological examination 26 cats from S. Kalimantan were found to be infected with B. pahangi, none with B. malayi and five with Dirofilariae repens. The DNA probes correctly identified all the B. pahangi infections but did not hybridize to any of the D. repens samples. In the 90 blood samples isolated from humans in S. Kalimantan only B. malayi mf were found by both techniques. The conclusion is that even in an area where there is a B. pahangi reservoir in the cat population there are very few if any B. pahangi mf found in humans.

B: FILARIASIS - EPIDEMIOLOGY AND IMMUNOLOGY

- 34 BLOCKING ACTIVITY LOCALIZES PREDOMINANTLY TO IGG4 ANTIBODIES IN BANCROFTIAN FILARIASIS. Hussain, R.\*; Poindexter, R. W. and Ottesen, E.A. Aga Khan University, Karachi, Pakistan, and Laboratory of Parasitic Diseases, National Institutes of Health Bethesda, MD.

'Blocking antibodies' (BA) that inhibit filarial antigen-specific, IgE-mediated histamine release from human basophils *in vitro* have been recognized at high levels in patients with bancroftian filariasis. The fact that this blocking activity can be completely removed by absorption of patient sera with either protein A or filarial antigen implies that it is a function of specific antibodies of one of the three IgG subclasses bound by protein A (IgG<sub>1</sub>, IgG<sub>2</sub> or IgG<sub>4</sub>).

When BA levels in the sera of 20 patients with bancroftian filariasis were compared with levels of specific anti-filarial antibodies in each of the four IgG subclasses, they correlated significantly only with IgG<sub>4</sub> antibodies ( $p < .01$ ). To confirm this localization of blocking activity to the IgG<sub>4</sub> subclass, three sera were depleted of IgG<sub>4</sub> (90-95%) by the anti-IgG<sub>4</sub> mouse monoclonal antibody 6023 bound to sepharose and were studied for their blocking capabilities in an *in vitro* assay in which normal basophils (stripped of autologous IgE) were passively sensitized with patients' IgE before being challenged with filarial antigen in the presence of the depleted or non-depleted sera. The depletion of IgG<sub>4</sub> reduced the blocking activity in these sera by 81%, 78% and 53%. As protein A absorption of these sera reduced the blocking activity by 91.2%, 99.9% and 94%, respectively, these results demonstrated that antibodies of the IgG<sub>4</sub> subclass appear responsible for the majority of blocking activity in the sera of patients with bancroftian filariasis but that a small contribution may also come from antibodies in the IgG<sub>1</sub> and IgG<sub>2</sub> subclasses.

- 35 IMMUNE RECOGNITION OF RECOMBINANT *ONCHOCERCA VOLVULUS* ANTIGENS. F.B. Perler, M.W. Southworth,\* I. Matsumura, M. Meda, and T.B. Nutman, New England Biolabs, Inc. Beverly, MA and Laboratory of Parasitic Diseases, NIH, Bethesda, MD.

Analysis of immune responses to *Onchocerca volvulus* (OV) infection, a leading cause of blindness in humans, is complicated by the spectral nature of the disease, a lack of parasite material, and broad antigenic cross reactivity among the various filarial parasites. The use of recombinant antigens overcomes several of these obstacles and provides defined materials for the study of the humoral and cellular immune responses to OV infection. Previous studies indicate that individuals potentially immune to OV infection exhibit modest antibody levels and a markedly elevated T cell response to soluble OV adult antigens, in contrast to those with active Onchocerciasis. In order to identify and isolate antigens responsible for inducing this immune state, OV genomic and cDNA expression libraries ( $\lambda$ gt11) were screened with defined patient sera and over 100 clones were selected. Characterisation of 7 of these clones indicates that 2 clones were preferentially recognised by all individual immune sera, but only by 4/9 disease sera; 4 clones selected with sera from immune patients were also recognised by all individual disease sera and 1 clone reacted with only 1 individual immune sera. These and other *Onchocerca* derived recombinant antigens should serve as monoclonal antigens for dissecting the immune responses to this important parasite.

36 T CELL CLONES AND LINES SPECIFICALLY RECOGNIZING  
ONCHOCERCA VOLVULUS ANTIGENS .

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Onchocerca volvulus (Ov), a filarial parasite of humans, is one of the world's leading causes of blindness. The T lymphocyte-mediated host immune response has been implicated in both the pathologic manifestations of onchocerciasis and possible immunity to this helminth. In order to examine the interaction between host T lymphocytes and parasite antigens, T cell lines (TCL) and clones with specificity for antigens of Ov have been generated using peripheral blood mononuclear cells (PBMC) from a patient naturally infected with Ov. TCL were generated by alternately stimulating PBMC with Ov adult antigen (OvA) in the presence of autologous feeder cells and expanding in media containing OvA and IL-2; T cell clones were isolated from these lines by limiting dilution and then expanded. 10 individual TCL and 11 clones produced in this manner exhibited marked proliferative responses to OVA with stimulation indices (SI) ranging from 5 to 153, while remaining non-reactive to the serologically cross-reactive filarial antigen preparation from Brugia malayi (BmA) (average SI=1.3). An additional 10 TCL and 5 T cell clones reacted with both OvA (SI range: 3.7 to 71) and BmA (SI range: 2.9 to 49.3), indicating their specificity for epitopes shared by these parasites. None of the clones or lines reacted to tetanus toxoid, an antigen to which the patient's PBMC are reactive. Identification of the antigens to which each of these TCL and clones react is currently underway using T cell immunoblotting. These clones and lines should prove useful for examining the immune response to Ov at the level of host T cell and parasite epitope interaction, and for defining species-specific antigens of Ov.

37 TWO COLOR FLOW CYTOMETRIC ANALYSIS OF LYMPHOCYTE PHENOTYPES IN ONCHOCERCIASIS. D.O. Freedman\*, A. Lujan, C. Gonzales, G. Zea-Flores, E.A. Ottesen, and T.B. Nutman. *Laboratory of Parasitic Diseases, NIH, Bethesda, Md. and the Ministry of Public Health, Guatemala City, Guatemala.*

Defects in the parasite-specific cellular immune response have been reported in individuals with onchocerciasis; however, data on the phenotypic profiles of circulating lymphocytes in affected individuals is lacking. To examine the relationship between the state of immune activation and lymphocyte phenotype in patients with onchocerciasis, dual color flow cytometry and concurrent *in vitro* peripheral blood mononuclear cell cultures were performed on microfilaridemic [MF; n=14] individuals from an endemic area of Guatemala and compared to normal [N; n=5] Guatemalans living in a non-endemic area. The ratio of helper/inducer to cytotoxic/suppressor (CD4/CD8) T-lymphocytes was not significantly different between the MF's and the N's (1.28 vs. 1.77). When the activation marker HLA-DR was examined, the number of 'activated' T-helper cells (CD4+DR+) was significantly increased in the MF's (10.67% of all CD4+ cells) when compared to the N's (6.19%; p<.005), while the expression of HLA-DR on cytotoxic/suppressor T- (CD8+) and B- (CD19+) lymphocytes did not differ between the two groups. The proportion of the helper T-cells that expressed the suppressor/inducer phenotype (CD4+CD45R+) was significantly higher in the MF group when compared to the N group (38.28 vs. 15.90%). Analysis of *in vitro* antigen and mitogen induced lymphocyte proliferation failed to show a correlation between stimulation indices to Onchocerca volvulus adult antigen, streptolysin O, PHA or ConA and the percentage of HLA-DR positive cells in any of the T-cell subsets or on B-cells. Another activation marker, the IL-2 receptor (CD25), was expressed equally in the two groups. These results provide the first data on the distribution of lymphocyte phenotypes in individuals with onchocerciasis and suggest a role for the suppressor/inducer subset of T-helper (CD4+CD45R+) cells in the immunoregulatory abnormalities seen in this disease.

## 8: FILARIASIS - EPIDEMIOLOGY AND IMMUNOLOGY

- 38 DOWN REGULATION OF T CELL GROWTH FACTOR PRODUCTION, BUT NOT RECEPTOR EXPRESSION IN BRUGIA PAHANGI INFECTED JIRDS  
L.E. Leiva\* and P.J. Lammie. LSU Medical Center, New Orleans, LA.

Previous studies have demonstrated that the induction of immunoregulatory mechanisms in the spleens of *B. pahangi* infected jirds is correlated with the onset of microfilaremia. The present study investigated the relationship between IL-2 production, IL-2 receptor expression, and the immunosuppression observed in the *B. pahangi*-jird model. A growth factor with IL-2-like activity (IL-2) present in culture supernatants of mitogen-stimulated jird lymphocytes supported the proliferation of murine CTLL cells and provided the basis for an IL-2 assay. Both *B. pahangi* antigen induced proliferative responsiveness and IL-2 production of spleen cells (Sp) from microfilaremic jirds were suppressed relative to lymph node cells (LN) from microfilaremic animals or Sp cells from *B. pahangi* immunized or prepatent jirds. IL-2 levels in 24 hr. culture supernatants of antigen stimulated Sp cells were 0.04, 1.7, 3.1, and 0.03 U/ml for normal, *B. pahangi* immunized, prepatent and microfilaremic jirds respectively. Depletion of histamine receptor bearing cells restored the ability of Sp cells from microfilaremic jirds to produce significant levels of IL-2 (from 0.08 to 0.9 U/ml). Further evidence that lymphokine production was actively regulated was obtained in add-mixture experiments. Sp cells from microfilaremic jirds suppressed antigen induced IL-2 production by cells from *B. pahangi* immunized jirds. An IL-2 binding assay was also used to quantitate levels of IL-2 receptor expression. Binding of labeled human IL-2 to high affinity receptors on jird lymphocytes was saturable (200 pM) and was specifically blocked by excess cold recombinant human IL-2 or concentrated supernatant from mitogen stimulated jird lymphocytes. IL-2 receptor expression was detected following *B. pahangi* stimulation of Sp and LN cells from *B. pahangi* immunized, prepatent or microfilaremic jirds. Despite an inability to proliferate in response to *B. pahangi*, Sp cells from microfilaremic jirds were capable of IL-2 receptor expression. This study demonstrates that the down regulation of immune responses in *B. pahangi* infection is a cell mediated event and is associated with an inability to produce lymphokines with IL-2-like activity, but not with an inability to express IL-2 receptors. (Supported by AI-24459).

- 39 BRUGIA PAHANGI AND BRUGIA MALAYI: A COMPARISON OF PATHOLOGIC AND IMMUNOLOGIC RESPONSIVENESS IN JIRDS. C.S. McVay\*, T.R. Klei, S.U. Coleman, S.C. Bosshardt and V.A. Dennis. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803

Jirds experimentally infected with *Brugia pahangi* have previously been used as models for pathogenesis studies of human lymphatic filariasis. In this study, host responses of jirds infected subcutaneously with the human parasite *Brugia malayi* were compared with those of jirds with infections of *B. pahangi*. Parasite burdens, lymphatic lesions, granulomatous reactivity to parasite antigen and antibody responses were assessed in jirds with infections of 60- and 150-day durations (DPI). At 60 DPI, the percentages of *B. malayi* adults recovered were markedly lower than those of *B. pahangi*; however, at 150 DPI, percentages of adult parasites recovered and microfilaremiacs were similar in both infections. Lymphatic lesion severity was maximal in both infections at 60 DPI and reduced at 150 DPI. At both time periods, however, animals infected with *B. pahangi* exhibited significantly greater lymphatic lesions. Granulomatous hypersensitivity to antigen-coated Sepharose beads embolized in the lungs was similar in both infections showing a significantly reduced response at 150 DPI. Jird eosinophil levels, and antibody responses exhibited similar patterns in both infections. Similarities in the kinetics of inflammatory reactivity in these infections suggest that previous findings on the *B. pahangi*-jird model could be utilized to design studies using *B. malayi* in this host. Further, the more marked lymphatic lesions observed in the *B. pahangi* infected individuals and the ease of maintaining this species support the continued use of this species as a conceptual model for studies on lymphatic lesion pathogenesis.

C: ANTIGENS OF LEISHMANIA AND TRYPANOSOMES

- 40 DEVELOPMENTAL MODIFICATION OF LEISHMANIA MAJOR LIPOPHOSPHOGLYCAN DURING METACYCLOGENESIS. D.L. SACKS\*, R. DA SILVA, AND S. TURCO†  
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†Department of Biochemistry, University of Kentucky Medical Center, Lexington, KY 40536.

During growth within the sand fly midgut and within axenic culture, Leishmania promastigotes undergo differentiation from a non-infective to an infective 'metacyclic' stage. For some species (e.g. L. major and L. donovani) this development is accompanied by loss of agglutination by the lectin peanut agglutinin (PNA). On non-infective, logarithmic phase promastigotes of L. major, PNA binds to the surface lipophosphoglycan (LPG). During metacyclogenesis, the LPG is developmentally modified such that it no longer binds PNA, it expresses a novel carbohydrate epitope, and it is substantially increased in size. The log and metacyclic LPGs were purified by organic solvent extraction and both were shown to be susceptible to PI-PLC and mild acid hydrolysis. The phosphorylated carbohydrate fragments released by mild acid were treated with alkaline phosphatase and analyzed by paper chromatography which revealed a difference in the tetrasaccharide subunits. The manner in which this structural change promotes complement resistance and intracellular survival is being investigated.

- 41 SEQUENTIAL MODULATION OF PROMASTIGOTE SURFACE MEMBRANE CARBOHYDRATES AND INFECTIVITY OF LEISHMANIA MAJOR.  
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Strains of L. major display a high degree of similarity in their enzyme electrophoretic profiles compared to other Old World species. However, they show considerable variation in promastigote surface membrane topography, determined by lectin specificities and released glycoconjugates. This surface and released glycoconjugate variation was monitored sequentially, using flow cytometry and crossed immunoelectrophoresis to identify factors pertaining to infectivity. Four L. major strains, two from Iran, one from the Jordan Valley and one from the Sinai, and four of its clones, were studied. The Iranian strains differed from each in their sequential labelling with fluorescent lectins (PNA, SBA and WGA) and their subserotypes and only one was infective to hamsters. Most of the Jordan Valley promastigote population was PNA positive (galactose) during the log and stationary phases of growth and was infective to hamsters. The Sinai strain and its clones showed different degrees of PNA and specific monoclonal antibody binding. All clones were uniformly virulent for outbred mice. Sugar and antigenic determinants were not clearly congruent with degrees of infectivity.

Acknowledgements This study was supported by contract No. NOI-AI-22668-NIH-NIAID and the leishmaniasis component of the UNDP/World Bank/WHO Special Programme TDR.

C: ANTIGENS OF LEISHMANIA AND TRYPANOSOMES

42 T CELL LINES WHICH TRANSFER PROTECTIVE IMMUNITY OR EXACERBATION IN CUTANEOUS LEISHMANIASIS BELONG TO DIFFERENT T HELPER SUBSETS.

P. Scott\*, P. Natovitz, E. Pearce and A. Sher. Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD 20892

Leishmania major infection in BALB/c mice leads to progressive lesion development and is ultimately fatal. We have shown that BALB/c mice can be protected against L. major infection by immunization with a partially purified, soluble sub-fraction of the parasite (fraction 9). In order to study the nature of the T cells contributing to either susceptibility or resistance in this model we established T cell lines reactive against both protective and non-protective leishmanial antigens. We demonstrate that a T cell line established against fraction 9, designated Line 9, transfers protection equivalent to that obtained by active immunization. In contrast, a T cell line (Line 1) responsive to a non-protective soluble fraction (fraction 1) not only failed to protect BALB/c mice against L. major, but exacerbated the infection. Most importantly, in addition to differing in their antigen specificity, protective and exacerbating T cell lines could be distinguished on the basis of the lymphokines produced, a characteristic previously used to separate murine T helper cells into 2 subsets, designated  $T_{H1}$  and  $T_{H2}$ . We found that the protective cell line, Line 9, displayed the  $T_{H1}$  property of secreting IL-2 and IFN- $\gamma$ , while the exacerbating lines secreted IL-4 and IL-5, a characteristic of  $T_{H2}$  cells. Our results demonstrate that  $T_{H1}$  and  $T_{H2}$  cells may have dramatically different effects on the outcome of an infection, and suggest that susceptibility and resistance in experimental leishmaniasis may depend upon a balance between the T helper subsets induced.

43 PURIFICATION AND PROPHYLACTIC IMMUNIZATION USING AN 80 KILODALTON PROTEIN FROM LEISHMANIA DONOVANI

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In an attempt to define an antigen for prophylactic immunization against visceral leishmaniasis, we initially focused on the antigen recognized by Mab D13. The epitope recognized by D13 is present in high titer on all L. donovani promastigotes by RIA and both promastigotes and amastigotes by IFAT. The antigen appears to be a nonglycosylated protein in that 1) it is readily labelled with [ $^{35}$ S]methionine, 2) Mab binding is not affected by periodate treatment, and 3) purified antigen does not bind radio-iodinated lectins. We have purified this antigen from L. donovani membranes by detergent solubilization, affinity chromatography with monoclonal antibody D13, and ion-exchange chromatography. In a pilot study, we immunized BALB/c mice i.p. with p80 (5  $\mu$ g) along with C. parvum (100  $\mu$ g) and boosted twice with p80 (2.5  $\mu$ g) plus C. parvum (50  $\mu$ g). Control groups received p80 alone, C. parvum alone, or PBS. The mice were subsequently infected with stationary phase promastigotes of L. donovani. Hepatic parasite burdens were determined from Giemsa-stained touch preparations quantitated as Leishman-Donovan units [(#amastigotes/#liver cell nuclei) x wt of liver(mg)]

Vaccine	Parasite Burden(LDU $\pm$ SEM)
PBS	1140 $\pm$ 164
<u>C. parvum</u>	1266 $\pm$ 81
P80	1061 $\pm$ 144
P80 + <u>C. parvum</u>	643 $\pm$ 52 ( $p < 0.05$ vs either PBS or <u>C. parvum</u> )

These results suggest that p80 is capable of eliciting protective immunity against L. donovani. Further studies are needed to find the optimal dose, route of administration, and adjuvant.

C: ANTIGENS OF LEISHMANIA AND TRYPANOSOMES

- 44 LEISHMANIA MAJOR: STRUCTURE OF GLYCOSYL-PHOSPHATIDYLINOSITOL ANTIGENS (GPI) RECOGNIZED BY IMMUNE HUMAN SERA. G.Rosen\*<sup>1</sup>, B.Nillson<sup>2</sup>, M.E. Westerman<sup>1</sup>, D.Sevlever<sup>1</sup> and M.V.Londner<sup>1</sup>. 1.The Kuvim Centre, Hebrew University-Hadassah Medical School, Jerusalem, Israel. 2.Bio Carb,Lund,Sweden.

We have previously reported the purification of three glycolipid antigens (GPI) (A, B, and C) from Leishmania major promastigotes which are able to bind immune human sera from patients with cutaneous leishmaniasis. The chemical structure of these GPI antigens has been elucidated by gas chromatography-mass spectrometry (GC-MS), and fast atom bombardment-mass spectrometry (FAB-MS). GC-MS of the alditol acetates revealed the presence of the following monosaccharides (molar ratio):

	Gal:	Man:	Glc	NAC:	Inos:	Glycerol
A	2	3	1	1	1	1
B	1	3	1	1	1	1
C	-	3	1	1	1	1

(All samples were acetylated before hydrolysis).

GC-MS analysis was also performed on the partially methylated alditol acetates. The positive-ion FAB spectrum of the permethylated glycolipids shows the following ions related to the Mr. A: m/z 2036 (M+H<sup>+</sup>), m/z 2058 (M+Na<sup>+</sup>); B: m/z 1746 (M+H<sup>+</sup>), m/z 1768 (M+Na<sup>+</sup>); C: m/z 1543 (M+H<sup>+</sup>), m/z 1565 (M+Na<sup>+</sup>). Fragment ions formed by cleavage across the glycosidic bonds allowed sequencing of the molecules. On the basis of these results, the structure of A, B and C is proposed as:

[Gal(1→3)]<sub>n</sub>Man(1→3)Man(1→3)Man(1→4)Glucosamine-alkylphosphatidylinositol.  
A:n=2; B:n=1; C:n=0.

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- 45 COMPARISON OF GLYCOLIPID ANTIGENS OF LEISHMANIA MAJOR AND LEISHMANIA DONOVANI PROMASTIGOTES. D. Sevlever\*, G. Rosen, M.E. Westerman and M.V. Londner. The Kuvim Centre, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

We have recently characterized glycolipid antigens of L.major (L.m.)<sup>1</sup>. In this study glycolipids from L.m. and L.donovani (L.d.) promastigotes were compared by biochemical and immunological methods. L.donovani donovani (L.d.d.) and L.m. promastigotes were extracted with hexane-isopropanol to yield a carbohydrate lipid fraction (CLF). After metabolic labeling with [<sup>32</sup>P] orthophosphate, [<sup>14</sup>C] mannose, [<sup>14</sup>C] glucosamine, [<sup>14</sup>C] inositol and [<sup>3</sup>H] myristic acid. L.d.d. and L.m. showed different patterns on thin layer chromatography (TLC). L.d.d. CLF was able to bind sera from patients with visceral leishmaniasis (VL) [causative agents: L.donovani infantum (L.d.i.) and L.d.d.] and from patients with cutaneous leishmaniasis (CL) (causative agent: L.m.) without any specificity when tested by ELISA. However, after immunoblotting of TLC, a band with Rf: 0.23 was recognized only by VL sera. This band was labeled by [<sup>14</sup>C] mannose, [<sup>14</sup>C] inositol, [<sup>14</sup>C] glucosamine and [<sup>32</sup>Pi] orthophosphate. When L.m. CLF, which reacted with CL sera, was tested by ELISA, 54% (12/22) of the L.d.d. sera were positive, this percentage dropped to 17% when TLC purified antigens of L.m. CLF were tested. None of the L.d.i. sera were found positive with CLF or with the purified L.m. antigens. This study shows that glycolipid antigens of L.m. and L.d. promastigotes are different in their chemical composition and serological reactivity.

1. G.Rosen, M.V.Londner, D.Sevlever and C.L.Greenblatt (1988). Mol.Biochem. Parasitol., 27, 93-100.

Supported by Contract N01-A1-2268-NIH-NIAID and Grant 86-00155 US-Israel BSF.

## C: ANTIGENS OF LEISHMANIA AND TRYPANOSOMES

- 46 DEVELOPMENT OF MONOCLONAL ANTIBODIES TO LEISHMANIA CHAGASI ANTIGENS WHICH CIRCULATE IN PERSONS WITH VISCERAL LEISHMANIASIS. T.G. Evans,\* M.J. Teixeira, B. Sutherland, Q. Boese, and R.D. Pearson. University of Virginia School of Medicine, Charlottesville, Virginia.

Sera of patients with visceral leishmaniasis contain immune complexes consisting of immunoglobulins and parasite antigens. These circulating antigens have potential diagnostic and immunologic significance. In order to obtain monoclonal antibodies against them, BALB/c mice were immunized with high molecular complexes precipitated from patient sera in 2.5% polyethylene glycol. Spleen cells from a mouse with a high antileishmanial antibody titer were fused to the sp/20 cell line. This resulted in 24 hybridoma lines that produced antibodies to L. chagasi as evaluated by ELISA.

Five hybridoma clones which produced antibodies to L. chagasi antigens (as determined by Western blot) were isolated by limiting dilution. Monoclonal 11C10 was of IgG2b isotype and recognized one antigen strongly at approximately 140 kDal and another weakly at 45 kDal. This monoclonal showed no reaction with control human serum. Periodate treatment (10 mmol) eliminated binding to the 140 kDal antigen suggesting that the monoclonal was directed against a glycoconjugate. Monoclonal 6H12 of IgM isotype detected a single 60 kDal antigen on reduced gels that migrated to approximately 40 kDal under non-reducing conditions. Periodate treatment reduced binding. This monoclonal reacted weakly to control human serum. Three other monoclonal antibodies gave complex banding patterns that could not be eliminated by using a variety of protease inhibitors. Monoclonals 11C10 and 6H12, which were produced against parasite antigens present in the sera of patients with visceral leishmaniasis, may be of use in the development of tests to detect, monitor, and characterize circulating antigens.

- 47 CHARACTERIZATION OF ANTIGEN EPITOPES OF INFECTIVE STAGE LEISHMANIA MAJOR PROMASTIGOTES BY USING MONOCLONAL ANTIBODIES IN WESTERN BLOT. S-J. Wu\*, E.D. Rowton, P.V. Perkins, M. Groggl and R.G. Andre. Department of Entomology, Walter Reed Army Institute of Research, Washington, D. C.

Sacks and Perkins (1984 and 1985) demonstrated that Leishmania promastigotes growing both in culture and within the sand fly vector developed from a noninfective to an infective stage. Monoclonal antibodies (McAbs) specific to the infective stage L. major promastigotes are needed for development of a rapid diagnostic assay to facilitate epidemiological studies and support future vaccine trials. Fifty-six McAbs reacting with L. major were developed from five fusions following an in vitro immunization method. Heterospecificity and stage-specificity tests were done for each positive McAb using both immunofluorescent antibody assay (IFA) and enzyme-linked immunosorbent assay (ELISA). Twelve McAbs showed specificity to L. major when tested against sympatric species of Leishmania. Four McAbs showed infective-stage specificity for L. major promastigotes. Characterization of antigen epitopes reacting with specific McAbs was carried out using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blotting and immunochemical staining. The total proteins of noninfective and infective stage promastigotes were each released and collected by sonification, detergent extraction and ultra-centrifugation. Proteins were separated by 10% SDS-PAGE, transferred by Western blotting, reacted with McAbs and immunochemically stained. Antigen epitopes unique to the infective stage promastigotes were identified.

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CARBOHYDRATE SPECIFICITY OF ANTI-HEART ANTIBODIES PRODUCED DURING EXPERIMENTAL CHAGAS' DISEASE IN MICE. T.S. McCormick\* and E.C. Rowland. Ohio University College of Osteopathic Medicine, Athens, OH.

C57Bl mice have recently been shown to develop anti-heart autoantibodies during *Trypanosoma cruzi* infection. At least some of these autoantibodies cross react with parasite extract. In an attempt to show that the target of these antibodies is the beta adrenergic receptor, turkey erythrocytes (TE), rich in these receptors, were found to bind these autoantibodies in ELISA assays. The work reported here extends the above results to examine the possibility that the target epitope(s) of autoantibody, from mice infected for 100 days, is carbohydrate in nature. In a competitive binding ELISA, both N-acetyl glucosamine and galactose were found to inhibit the binding of the antisera to heart extract, TE and sheep erythrocytes in a dose dependent manner to a maximum of about 40% inhibition. Similarly, binding of antisera to *T. cruzi* extract was inhibited by these carbohydrates. Mild acid hydrolysis treatment was used to cleave carbohydrate moieties from heart extract proteins and treated extracts were found to bind less autoantibody than untreated heart extracts. The effect of carbohydrate specific enzyme treatment of heart and parasite extracts on their ability to react with autoantibodies will be presented. These results suggest that a portion of the autoantibody response in chagasic mice is directed to carbohydrate epitopes which may be common on unrelated glycoproteins. (Supported by NIH grant AI-23704).

49 TRYPANOSOMA CRUZI INVADE MAMMALIAN EPITHELIAL CELLS IN A POLARIZED MANNER. S. Schenkman, V. Nussenzweig and E.S. Robbins. N.Y.U. Medical Center, New York, NY.

In order to determine whether parasite entry into host cells can be influenced by cell polarity we studied the infection of Madin-Darby canine kidney (MDCK) cells by *Trypanosoma cruzi*. When confluent these cultured epithelial cells are in close contact and highly polarized with junctional complexes which are thought to maintain the separation of apical and basolateral domains. Both trypomastigotes released from cultured mammalian cells and metacyclic forms derived from cultured epimastigotes avidly infected sparsely plated MDCK cells but were almost unable to infect the same cell type when the cultures were confluent. The infection was measured by hybridization with a parasite specific DNA probe. When *T. cruzi* had access to the basolateral side of confluent monolayers through porous filters they more readily infected the cells. If confluent MDCK monolayers were incubated in  $Ca^{2+}$  free medium containing EGTA the cells changed shape and intercellular junctions were disrupted. *T. cruzi* were much better able to infect such disrupted confluent monolayers. After wounding confluent monolayers were infected by trypomastigotes and scanning electron microscopy (SEM) examination showed that the only cells being entered by parasites were adjacent to the wounds. Sparsely plated epithelial cells are partially polarized and when examined by SEM 92 ± 4% of the parasites were seen entering at the edges of such non-confluent cells. These experiments demonstrate that epithelial cell polarity limits *T. cruzi* invasion. In addition, parasite polarity is also an important aspect of infectivity with 76 ± 6% of parasites entering cells by their posterior, ie flagellar end.

C: ANTIGENS OF LEISHMANIA AND TRYPANOSOMES

- 50 DEVELOPMENTALLY REGULATED TRYPANOSOMA CRUZI TRYPOMASTIGOTE  
83 KD GLYCOPROTEIN (GP) BINDS TO MAMMALIAN HOST CELLS IN  
A LIGAND RECEPTOR INTERACTION MANNER. M.F. Lima\* and F. Villalta.  
Division of Biomedical Sciences, Meharry Medical College, Nashville, TN.

Trypanosoma cruzi trypomastigotes must attach to mammalian cells before they can invade them. An understanding of this process is critical to the development of effective means to prevent the infection. We have identified an 83 gp on the cell surface of trypomastigote clones that mediates the binding of trypomastigotes to heart myoblasts by western blot. The binding of biotinylated 83 gp to myoblasts is inhibited by excess of unlabeled counterpart in western blots, as indicated by laser scanning densitometry. In addition, binding of iodinated 83 gp to heart myoblasts is saturable and inhibitable by cold excess. The 83 gp is not present on the cell surface of non invasive epimastigotes, but it is expressed in trypomastigotes arising when epimastigotes differentiate into trypomastigotes. This molecule is differentially expressed in distinct populations of trypomastigote clones with different degrees of cellular invasiveness, indicating that the 83 gp is not only developmentally regulated in stages of the parasite cycle, but also in subpopulations of trypomastigotes. There is a direct correlation between attachment or invasion of host cells by culture trypomastigote clones with different degrees of 83 gp expression on the surface of trypomastigotes. Antibodies against the surface of insect trypomastigotes, blood trypomastigotes as well as those produced during human infection recognize the 83 gp adhesion molecule by immunoblotting, indicating that this adhesion molecule is immunogenic and is a candidate for vaccination against Chagas' disease. (Supported by USAID grant DAN-5053-G-SS-7076-00).

- 51 An acid pH-dependent hemolysin from Trypanosoma cruzi  
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Center, New York, N. Y.

Trypanosoma cruzi releases into the medium a heat labile, trypsin sensitive agent which is lytic for erythrocytes from several species. Production of the active hemolysin could be abolished by removal of glucose from the medium or by addition of the metabolic inhibitors sodium azide, 2-deoxyglucose or puromycin. Sieving experiments with erythrocyte ghosts indicated that large lesions were being formed on the target membranes. The fact that the activity was maximal at pH 5.5 and undetectable at neutral pH suggests a role for this hemolysin in acidic intracellular compartments. It is conceivable that this molecule is involved in promoting the escape of T. cruzi into the cytoplasm of the host cell, through disruption of the phagosome in which this parasite resides at early times after invasion.

C: ANTIGENS OF LEISHMANIA AND TRYPANOSOMES

- 52 A PROTEIN MARKER FOR ENDOCYTOSIS IN TRYPANOSOMA BRUCEI.  
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on Animal Diseases (ILRAD), Nairobi, Kenya. Present address: Dept.  
of Veterinary Pathobiology, University of Illinois, Urbana, IL.

Endocytosis occurs in African trypanosomes by the formation of spiny-coated vesicles. Coated vesicle endocytosis in eukaryotes reflects receptor-mediated endocytosis of many biologically important molecules. African trypanosomes have been reported to take up two nutrients, low-density lipoprotein and transferrin, by the process of receptor-mediated endocytosis. Little is known about the nature of the parasite receptors for these molecules or other host molecules that might be selectively endocytosed. To study receptor-mediated endocytosis by trypanosomes, coated vesicles from bloodstream form T. brucei were purified and characterized by electron microscopy and SDS-PAGE. Integral membrane proteins were purified from the coated vesicles by phase separation with Triton X-114. The largest major membrane protein was a doublet band with a molecular mass of about 77kDa. A monospecific antiserum was prepared against this protein by immunization with antigen bands excised from SDS-PAGE gels. Using this serum in immunoblots it was discovered that the 77kDa protein is absent from the procyclic stage of the parasite found in the midgut of the tsetse fly; this stage does not appear to engage in coated vesicle endocytosis. Immunofluorescent studies localized the antigen in the region between the flagellar pocket and the nucleus of bloodstream forms. Ultra-structurally, the antigen was detected on membranes of endosomes and lysosome-like structures that contained endocytosed markers. This protein appears to be a specific marker for endocytotic vesicles and may be functionally involved in receptor-mediated endocytosis by the parasite.

EFFECTIVE ADVOCACY WORKSHOP: 1989 ASTMH POLITICAL ACTION PLAN

- 53 K.M. Johnson. Former President, ASTMH.
- 54 S.F. Kuvin. Committee for Public Affairs and Task Force for Political Action, Palm Beach, FL.
- 55 W.E. Small. Executive Director for National Foundation of Infectious Diseases, Bethesda, MD.
- 55a Congressional Staff (to be identified).

ASTMH POSTER I

- 56 VARIATION IN SEASONAL PREVALENCE RATES OF NEUTRALIZING ANTIBODY TO JAMESTOWN CANYON VIRUS IN THREE WHITE-TAILED DEER POPULATIONS AND VECTOR COMPETENCE OF SELECT MOSQUITO SPECIES POTENTIALLY ASSOCIATED WITH THE NATURAL TRANSMISSION CYCLE IN THE UPPER MIDWEST.

Paul R. Grimstad, University of Notre Dame, Notre Dame, IN

To better understand the natural transmission cycle of Jamestown Canyon (JC) virus producing a bimodal period of case onset in humans in the Upper Midwest, New York and Canada, we have used white-tailed deer as sentinel animals in northern Indiana and Michigan. Continued blood sampling of one captive breeding herd year-round and two feral populations during fall hunting season indicate that seroconversion occurs coincident with the emergence of spring broods of Aedes mosquitoes. However, the level of herd immunity realized, as measured by neutralizing antibody in Vero cell culture, is affected by the relative Aedes populations each spring. Transmission trials using JC virus have been used to evaluate the vector competence of Aedes stimulans, Anopheles punctipennis, Mansonia perturbans and other species. Oral transmission rates of JC virus by Ae. stimulans vary little among regional geographic populations while the major variation seen with An. punctipennis is seasonally related. M. perturbans may be regionally important in bridging the spring vs. late summer transmission periods. The results of the deer seroconversion and mosquito vector competence studies suggest a possible model for the bimodal case onset that involves multiple vector species in different geographic regions. The effect of one season's record low Aedine population (simulating a mosquito control effort at vector control) on herd immunity and fawn maternal antibody levels will be presented along with the rebound associated with normal transmission rates in the spring of 1988.

- 57 THE POTENTIAL IMPACT OF MULTIPLE BLOOD-FEEDING BY CULISETA MELANURA ON THE EPIDEMIOLOGY OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS.

R.A. Anderson,\* J.D. Edman, T.W. Scott, and L. Lorenz. University of Massachusetts, Amherst, MA and University of Maryland, College Park, MD.

The relative frequency and success of blood-feeding behavior of mosquitoes is an important factor underlying disease transmission. The goal of this research project is to examine the hypothesis that Culiseta melanura engage in multiple probing and blood-feeding on closely related hosts and that this behavior significantly increases the vectorial capacity of this species for Eastern Equine Encephalomyelitis (EEE) Virus. This research project encompasses 2 main objectives: 1) to evaluate the multiple feeding rate of Culiseta melanura in an enzootic focus of EEE virus transmission. 2) to evaluate the potential of Culiseta melanura to transmit EEE virus more than once per gonotrophic cycle in the lab. Rubidium and cesium are 2 alkali metals that have been evaluated in a preliminary manner as host-blood markers for investigating multiple blood-feeding the field. A blood marking technique using these alkali metals has been used to demonstrate a multiple feeding rate of 13% (5/39 blood-meals) by wild Culiseta melanura on caged chickens in the Hockamock swamp in MA. Although no method currently exists for measuring multiple probing events in the field, 4 of 11 Culiseta melanura which were intrathoracically inoculated with EEE virus transmitted the virus without visible engorgement to susceptible chicks. The implications of multiple probing and blood-feeding by EEE infected Culiseta melanura to the epidemiology of enzootic transmission is discussed.

58 MULTIPLE HOST CONTACTS BY CULISETA MELANURA AND TRANSMISSION OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS

T.W. Scott,\* L.H. Lorenz, S.C. Weaver, R.A. Anderson, J.D. Edman, and W.S. Romoser. University of Maryland, College Park, MD, University of Massachusetts, Amherst, MA, and Ohio University, Athens, OH.

We are studying the eastern equine encephalomyelitis (EEE) virus transmission cycle to determine the contribution of multiple host contacts by mosquitoes to virus transmission. Transmission after a brief extrinsic incubation (EI) period or multiple transmissions by a single mosquito would significantly increase the probability of vertebrate infection. We began by using a histological technique to examine engorged Culiseta melanura for peritrophic membrane (PM) deposition. Laboratory experiments showed that a minimum of 10 hr between blood meals is necessary to detect multiple feeding. The first sign of PM deposition was seen by electron microscopy 6 hr after feeding. PM material appeared fully formed by 12 hr. To date 203 of >800 engorged Cs. melanura collected in the field have been examined. No multiple meals were detected. In order to detect multiple meals separated by less than 10 hr, a technique was developed using alkali metals as host blood markers. During a field trial, 13% (5/39) of the Cs. melanura that fed on marked chickens imbibed double blood meals. Laboratory studies were carried out to assess the ability of mosquitoes to transmit virus under these conditions. Intrathoracically inoculated Cs. melanura transmitted EEE virus to chickens (4/11) without imbibing blood. We have not been able to demonstrate mechanical transmission (n=34). These data suggest that Cs. melanura do not transmit EEE virus after 2-3 days of EI, but infected mosquitoes may transmit virus more than once during a single gonotrophic cycle.

59 MAINTENANCE OF Aedeomyia squamipennis - A LABORATORY MODEL OF TRANSOVARIAL TRANSMISSION.

J. L. Petersen,\* B. E. Dutary, and P. H. Peralta.  
Gorgas Memorial Laboratory, Panama.

We have shown previously that the mosquito Aedeomyia squamipennis transovarially transmits Gamboa virus (Bunyaviridae). We hypothesized that transovarial transmission (TOT) is the primary mechanism by which Gamboa virus is maintained in nature, and that viral amplification in vertebrates is of secondary importance. The evidence supporting this hypothesis was based on natural infection rates determined from field data. An impediment to critically testing our hypothesis was the lack of a laboratory colony of Ad. squamipennis. We now report the laboratory maintenance of Ad. squamipennis from egg to adult. Egg clutches were collected in the natural habitat, isolated in 250 cc filtered river water and hatched in the laboratory. First instar larvae were separated into groups of fifty or ten and placed in 2-liter screened plastic containers provided with 50% cover of the floating aquatic fern Salvinia auriculata. We recorded mean development time from hatch to pupation (41 days), per cent survival to the pupal stage (60%), mean male wing length (2.02 mm), and mean female wing length (2.08 mm). Mean adult wing length (an indication of overall fitness) of lab-reared Ad. squamipennis was not significantly different from the mean wing length of field-collected adults. Results of progeny-testing infected lab-reared Ad. squamipennis will be reported.

ASTMH POSTER I

- 60 Effect of Immune Bloodmeals on the Vector Competence of Aedes triseriatus for La Crosse Virus. M.S. Godsey, Jr.,\* G.R. DeFoliart and T.M. Yuill. Departments of Entomology and Veterinary Science, and School of Veterinary Medicine, University of Wisconsin, Madison, WI.

Recent evidence suggests that transmission of La Crosse virus (LACV) by Aedes triseriatus mosquitoes may be adversely affected by ingestion of blood containing antibody to LACV or the related Jamestown Canyon virus (JCV). Engorgement on LACV-immune chipmunks caused a 40% reduction in oral transmission of LACV by venereally infected A. triseriatus. Mosquitoes engorging through a membrane feeder on chipmunk blood containing LACV and LACV antibody, or deer blood containing LACV and JCV antibody had infection rates similar to controls, but oral transmission was reduced or inhibited.

To further explore this phenomenon we have fed groups of A. triseriatus on LACV-chipmunk blood mixtures in a membrane feeder. Following oviposition a second bloodmeal was taken on LACV- or JCV-immune chipmunks or deer, or on non-immune controls. After the second oviposition females were fed individually on suckling mice to determine oral transmission rates. After this bloodmeal females were tested for LACV by ELISA. Eggs collected from the second and third ovipositions were hatched, and adult progeny tested for LACV by ELISA to determine transovarial transmission rates. The implications of our findings for the distribution and enzootic maintenance of LACV will be discussed.

- 61 SITES OF RIFT VALLEY FEVER VIRUS INFECTION IN THE PROVENTRICULUS OF ADULT CULEX PIPIENS. K. Lerdthusnee and W. S. Romoser, Ohio U., Athens, Ohio

Earlier studies of dissemination of Rift Valley fever virus from the adult midgut suggested that this virus can infect cells at the foregut-midgut junction within the proventriculus. In order to identify sites of infection more precisely, we studied the structure and viral infection of the proventriculus in Cx. pipiens using optical and electron microscopy and immunocytochemistry.

The proventriculus forms as an intussusception of foregut and midgut epithelia. The outer layer is midgut tissue (the cardiac epithelium). Inner layers consist of esophageal epithelium which enters the cavity formed by the cardiac epithelium and turns ectad and then anteriorly, forming the reflected wall. Inserted between the anterior end of the cardiac epithelium and the termination of the esophageal epithelium is a ring of cells (R1 cells). In larvae, non-cellular intima ends with its attachment to the R1 cells. A remnant of this intima in the adult forms an irregular cone which points posteriorly into the midgut lumen. The adult foregut intima appears to end on the reflected wall of the esophagus. Our studies provide evidence that sites of virus infection from the lumen are in the region where the reflected esophageal epithelium and R1 cells join.

- 62 CONTROL OF GENE EXPRESSION IN THE SALIVARY GLANDS OF VECTOR MOSQUITOES.  
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The recent demonstration of transformation in vector mosquitoes has provided the means for introducing hybrid genes designed to interfere with the development and replication of parasites. Our efforts are focused on developing expression of hybrid genes in specific tissues of mosquitoes. Towards that end, we are isolating and characterizing genes expressed specifically in the salivary glands of various vector mosquitoes. Our analysis includes the isolation and sequencing of genomic and cDNA clones, determination of their expression patterns through development and in tissues, and identifying the sequences that control the tissue-specific expression. Initially, the control sequences are linked to a marker gene to determine the specificity of expression of the hybrid gene. The marker gene is then replaced with a specific DNA fragment chosen to interfere with a specific pathogen. We report here on the results of our efforts using two genes expressed in different regions of the salivary glands of *Aedes aegypti*. One of these genes is also present in other vector mosquitoes. This gene encodes an abundant mRNA that is transcribed in the cells of the proximal lateral lobes of the female gland and the deduced protein displays striking similarities with the products of a yeast maltase gene and three previously unidentified genes from *Drosophila melanogaster*. The presumed function of this gene product is to assist the mosquito in its sugar-feeding capabilities. The second gene has a different expression pattern and its product may be involved in bloodfeeding. The control sequences of both genes are being used to direct the expression of a marker gene in transformation studies.

- 63 BINDING OF WEE VIRUS TO BRUSH BORDER FRAGMENTS ISOLATED FROM THE  
MESENTERONAL EPITHELIAL CELLS OF SUSCEPTIBLE AND REFRACTORY CULEX  
MOSQUITOES.

Edward J. Houk\*, Yvonne M. Arcus and James L. Hardy. School of Public  
Health, University of California, Berkeley CA 94720.

It has been suggested that the mesenteron infection barrier to arboviral infection of refractory mosquitoes may be a consequence of the absence of, or the alteration of, specific receptor sites. This hypothesis was examined by studying the interaction of radiolabeled WEE virus with brush border fragments isolated from dissected mesenterons of both susceptible and refractory Culex mosquitoes. Briefly, 5-10 ug of brush border fragments and an appropriate amount of radiolabeled WEE virus were mixed in a microcentrifuge tube, allowed to incubate for 1 hr and bound WEE virus was pelleted with brush border fragments at 27,000xg-30min.

Optimal binding occurred at pH 7.2 and 20°C. There was no apparent requirement for divalent cations. Saturation and competitive binding experiments clearly demonstrated the specificity of the reaction in susceptible mosquitoes. Scatchard analysis of the binding data resulted in an estimate of the number of binding sites/mesenteron epithelial cell at  $1.7-3.4 \times 10^6$ . The affinity constant ( $K_a$ ) was determined to be  $2.3 \times 10^{11} M^{-1}$ .

ASTMH POSTER I

- 64 SEASONAL ABUNDANCE OF DENGUE VECTORS IN MANILA, REPUBLIC OF THE PHILIPPINES. George W. Schultz\*. U.S. Naval Medical Research Unit No. 2, Manila, Philippines.

The dengue vectors, Aedes aegypti and Ae. albopictus, were surveyed in the city of Manila to determine seasonal abundance and distribution. Survey techniques included ovitraps, indoor resting collections, Fay traps, larval surveys, and man-biting collections. Aedes aegypti was the principal container breeder within the residential areas, while Ae. albopictus dominated in areas with abundant vegetation. The ovitrap was very effective in detecting the presence of Ae. aegypti, but not in measuring population changes during the year. Indoor resting and Fay traps show a low population of Ae. aegypti from February through May, during the dry season, and higher populations from June through September, during the rainy season. The most common indoor containers holding larvae were flower vases, while metal or glass containers were the most common outdoors. Biting activity of both species is similar, with peaks occurring between 0530-0600 and 1730-1800. Dengue transmission appears to be closely related to rainfall, with cases increasing about 2 months following the onset of the rainy season.

- 65 Failure of Ultra-low Volume Insecticide to Penetrate Typical Resting Sites OF Aedes aegypti. P. Reiter,\* M.A. Amador and D.J. Gubler, Dengue Branch, Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, GPO Box 4532, San Juan. Puerto Rico 00936

Dengue and dengue hemorrhagic fever/dengue shock syndrome are evolving as a major public health problem in the Americas. Control of the adult vector, Aedes aegypti, by ultra low volume (ULV) insecticide is the emergency measure most frequently used during epidemics. Previous assessments of the efficacy of ULV, which indicated satisfactory control, generally relied on the mortality of caged mosquitoes placed at exposed sites in the treatment area. However, extensive trials conducted recently in San Juan, Puerto Rico, using a surveillance method that directly monitors the impact of the fumigant on wild Ae. aegypti, repeatedly demonstrated that the treatment method was ineffective. We believe the principal reason for this discrepancy must be a lack of interaction between the aerosol and the wild adult mosquitoes. In a series of ten trials we assessed dispersal of the insecticide by measuring mortality in bioassay cages suspended at 42 sites in the domestic environment, and by spinning droplet collectors. Test mosquitoes included laboratory (fully susceptible) and local (slightly tolerant) strains, as well as local strains in the unfed, engorged and gravid condition. Results consistently demonstrated that the insecticide penetrates indoors but that mortality is more dependent on the immediate placement of the cage than on whether it is indoors or outdoors. Mortality was especially low in cages placed in sheltered locations representative of typical resting sites of adult Ae. aegypti (see Clark, Seda & Gubler). We conclude that ULV as used at present is inappropriate for the control of this species.

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- 66 INTRADOMICILIARY ACTIVITY OF Aedes aegypti IN SAN JUAN, PUERTO RICO. G.G. Clark,\* H. Seda, and D.J. Gubler. Dengue Branch, Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, GPO Box 4532 San Juan, Puerto Rico 00936

During epidemics of dengue or other Aedes aegypti-borne viral diseases, public health officials must rely on vehicle-mounted or aerial application of ultra-low volume (ULV) of insecticide for the immediate elimination of virus-infected females. Results of recent studies of the efficacy of adult mosquito control techniques in Puerto Rico have indicated that insufficient indoor penetration of insecticide may be one of the reasons that natural Ae. aegypti populations are not significantly reduced. Using a large, battery-powered aspirator, we have made weekly indoor collections of adult mosquitoes in a group of houses in a lower-middle class urbanization of San Juan. During the first 13 weeks of the study, we collected 4,446 (2,091 males and 2,355 females) Ae. aegypti. Three of the most productive houses averaged over 40 mosquitoes per week. Female Ae. aegypti were found in all developmental stages from recently emerged to fully gravid. Seventy percent of female mosquitoes were collected from bedrooms and most of these were recovered from closets. The recovery of this large percentage of mosquitoes from secluded indoor resting sites is important in explaining the apparent lack of efficacy of extradomiciliary ULV fumigation. These results, if verified in other urban areas, present an additional obstacle for controlling dengue epidemics.

- 67 FIELD EVALUATIONS OF A CLOTHING IMPREGNANT AND THREE TOPICAL REPELLENT FORMULATIONS AGAINST TSETSE FLIES IN ZAMBIA. L.L. Sholdt,\* C.E. Schreck, M.I. Mwangelwa, J. Nondo, V.J. Siachinji. Uniformed Services University of the Health Sciences, Bethesda, MD; USDA Insects Affecting Man and Animals Laboratory, Gainesville, FL; Tropical Disease Research Centre, N'Dola, Zambia and Livestock Pest Research Centre, Chilanga, Zambia.

Clothing impregnated with permethrin (a synthetic pyrethroid) and three topical repellent formulations of deet (diethyl methylbenzamide) were tested in the field against populations of tsetse flies, mostly Glossina morsitans centralis, in central Zambia. Different combinations of impregnated clothing and repellents were worn by volunteers riding in an open vehicle driven slowly through fly-infested areas. The treatment combination of permethrin-impregnated clothing and either of two controlled-release deet formulations provided 91% protection. Equally effective by statistical significance was wearing either of the controlled-release repellents alone (80 and 87% protection). Wearing impregnated clothing only provided relatively poor protection for the untreated and exposed skin (34%). Mesh jackets treated with permethrin, but without repellent on exposed skin, were 75% effective in preventing tsetse fly bites. Improved repellent formulations of deet at about one-half the dosage of the active ingredient, provided up to 10% more protection from tsetse fly bites than did 75% deet in ethanol. Wearing adequate clothing provides additional protection because it serves as a physical barrier to delay bites. When the clothing is treated with permethrin, flies on contact quickly absorb enough of the chemical to cause knockdown or other effects that inhibit biting.

ASTMH POSTER I

- 68 IDENTIFICATION AND GENETIC CHARACTERIZATION OF CERTAIN VERRUCARUM GROUP spp. (DIPTERA: PSYCHODIDAE: LUTZOMYIA). R.D. Kreutzer\*, T.M. Palau, A. Morales, C. Ferro, D. Feliciangeli and D.G. Young. Youngstown State University, Youngstown, OH, Instituto Nacional de Salud, Bogota, Colombia, Universidad de Carabobo, Maracay, Venezuela and University of Florida, Gainesville, FL.

Within the Verrucarum group of species in the genus Lutzomyia which include many proven vectors of leishmaniasis the females of many species are difficult, if not impossible (isomorphic), to identify without associated males. Thus, infected females from a mixed population often cannot be specifically identified. A study was made of isozyme data (21 gene loci) from field populations of the isomorphic female species L. spinicrassa, L. youngi, L. townsendi, L. longiflocosa, L. quasitownsendi, L. sauroida. Data from 6PGDH and FUM will separate L. youngi and L. spinicrassa add data from PFK, PGM and GPI and L. townsendi would be separated as would the remaining species trio (themselves unseparable by the enzymes studied). Data concerning specific identification of dissected sand flies will also be presented.

An analysis of genetic identities (I) and distances was also made. The results suggested that L. longiflocosa, L. quasitownsendi, L. sauroida might be conspecific populations ( $I = 0.991$ ) and that the others have diverged enough to be considered sub- or incipient species ( $I = 0.774$  to  $0.853$ ). The genetic analysis was extended to include L. serrana, L. columbiana and L. andina. Relationships among all the species will be discussed as will data on average polymorphic loci ( $P = 19-38\%$ ) and heterozygosity ( $H = 8-11\%$  expected and  $7-10\%$  observed).

Supported by Grant #AI-20108, National Institutes of Health.

- 69 Population structure of the Egyptian Culex pipiens complex (Diptera: Culicidae) A.M. Gad and Hassan A.N.\*

Culex pipiens the vector of filariasis and several arboviruses in Egypt have commonly reported to exist in two forms; autogenous and anautogenous. Attempting to determine the structure of this mosquito in filarial endemic and nonendemic areas we demonstrated that besides the autogenous and anautogenous a third form "intermediate" readily exists. Ovarian examination and host seeking experiments disclosed that in intermediate females, ovarian development is arrested (without atresia) at Christophers's stage IIB-III 2 days post emergence after such time those individuals seeked host, imbibed blood and oviposited similar to parous autogenous and nulliparous (Christophers's II) anautogenous siblings. Field observations indicated also that one third of the baited traps catches were made of nulliparous intermediate females. The 3 Cx. pipiens forms share enclosed and open breeding sites and are stenogamous. Culex pipiens populations derived from hypogeal habitats contained 2-81%, 0.0-90% and 0.0-97% (n = 748) while those of epigeal origin contained 2-55%, 0.0-82% and 12-98% (n = 600) intermediate, autogenous and anautogenous individuals respectively. In conclusion, we defined 3 distinct forms of Cx. pipiens in Egypt and recommended that future determination of population structure should incorporate those 3 forms. Finally, the question of "what form(s) of Cx. pipiens vector filariasis and other arboviral diseases" still remains.

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INTENSE TRANSMISSION OF THREE MALARIA SPECIES BY ANOPHELES MACULATUS  
AMONG ORANG ASLI IN EASTERN PERAK, PENINSULAR MALAYSIA.

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An ongoing study of an intense focus of endemic malaria in aboriginal (Orang Asli) settlements in a low mountainous valley is described and analyzed entomologically. Malaria endemicity in over 500 inhabitants of 16 villages averaged between 37-40%, as determined by 10 point-prevalence studies between August 1986 - May 1988. Over 9,000 man-hours of all night mosquito collections made in 6 of the villages during this period resulted in over 3,000 captured and dissected anophelines of 7 species. Anopheles maculatus constituted 93.5% of all anophelines collected, and was the only species found gland positive for Plasmodium species. Gland positive rates in maculatus ranged from 0 to 20.5% in 36 entomological surveys, and represent the highest gland positive rates recorded for maculatus. Positive maculatus salivary glands and associated head-thorax components were analyzed for Plasmodium species identification by the ELISA. The blood source of engorged An. maculatus was identified by the DOT-ELISA. In addition the ecology of the study area is discussed in terms of man-vector contact, with emphasis on vector biting behavior, vector parity rates and estimates of sporozoite inoculation rates.

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NEW WING CHARACTERS FOR THE IDENTIFICATION OF ANOPHELINE VECTOR  
GROUPS

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A standardized nomenclature for the costal wing spots of genus Anopheles and other spotted wing mosquitoes is presented. Emphasis is given to An. (Anopheles) Series Arribalzagia which are found to have 3 unique costal regions. An isolated dark spot at the end of the subcostal vein in one of these regions serves to define this group. Several fixed morphological markers on the wing are used to establish reference points for the definition of the other costal spots. Better definition of the costal wing spots in Anopheles mosquitoes enhances their value as characters to be used in identification and classification of vector groups.

ASTMH POSTER I

- 72 EPIDEMIOLOGY OF HEPATITIS B VIRUS AND SEXUALLY TRANSMITTED DISEASES AMONG PROMISCUOUS HETEROSEXUALS IN SUDAN  
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In order to determine the prevalence and risk factors for transmission of hepatitis B virus (HBV) and sexually transmitted diseases (STD) among high-risk groups in Port Sudan, a seroepidemiological study was conducted among 203 female prostitutes, 263 male customers of prostitutes, 62 female controls and 168 male controls in March and October, 1987.

In comparison to female controls, the prostitutes had a higher prevalence of anti-HBs (60% vs. 18%;  $p < .001$ ), total HBV markers (HBsAg, anti-HBs or anti-HBc) (83% vs. 52%;  $p < .001$ ), HBeAg (09% vs. 0%), antibody to T. pallidum (30% vs. 08%;  $p = .001$ ), and antibody to C. trachomatis (94% vs. 73%;  $p < .001$ ). When compared to male controls, the customers of prostitutes had a higher prevalence of anti-HBc (20% vs. 12%;  $p = .01$ ), HBeAg (11% vs. 06%), antibody to T. pallidum (15% vs. 08%;  $p = .009$ ), and a history of STD (65% vs. 44%;  $p < .001$ ). An association between HBV markers and STD was not noted.

This study demonstrated that the prevalence of HBV and STD are higher among prostitutes and their customers in Port Sudan, and that these groups represent infectious reservoirs of HBV and STD. In addition, it suggests that sexual transmission of HBV may be significant in this population, and that HIV-1 has the potential to disseminate since the modes of transmission are similar to HBV.

(Supported by NMRDC Bethesda, MD., Work Unit No. 3M161102.BS10.AK311)

- 73 A SEROSURVEY OF HEPATITIS MARKERS IN THE YEMEN ARAB REPUBLIC.  
D.A. Scott, J.P. Burans, K.C. Hyams, H.D. Al-Ouzeib, A. Al-Hadad, M. Al-Faddel, Y.R. Nigad, B.K. Arunkumar, J.N. Woody. US NAMRU-3 Cairo, Egypt and Ministry of Health, Sanaa, Yemen Arab Republic.

Viral hepatitis and its sequelae are significant world health problems. Much remains unknown about the incidence and routes of transmission. Therefore, during February 1988, a serosurvey was conducted to determine the prevalence of viral hepatitis markers and risk factors associated with their acquisition in the Yemen Arab Republic. Random serum specimens and epidemiologic information were collected in the four main population centers; Sanaa, Hajja, Hodeida, and Taiz. All sera were screened for HBsAg and HAVIgG by ELISA. Sera negative for HBsAg were screened for anti-HBs and/or anti-HBc, and positive sera for the presence of anti-Delta. A total of 905 sera were collected, 886 had adequate volume for all testing. Seven sera from Ibb were excluded from risk factor analysis. Of the total, 112 (13%) were positive for HBsAg and 401 (45%) were positive for at least one hepatitis B marker. Only 2 (0.2%) sera were positive for anti-Delta. Over 99% were positive for HAVIgG. Increasing age was significantly associated with HBsAg status ( $p < .05$ ). Age and prior surgery were both associated with total markers ( $p < .05$ ). Blood transfusion was significant on univariate analysis ( $p = .025$ ), but the significance dropped ( $p = .070$ ) when controlled for operations.

This survey indicates that hepatitis B infection is a significant health concern in the Yemen Arab Republic. The association of surgery and possibly blood transfusions point out routes of transmission that are preventable. The rates of hepatitis A are as expected.

Supported by NMRDC Bethesda, MD. Work Unit Number 3M16102.BS13.AK31

ASTMH POSTER I

- 74 A CROSS-SECTIONAL SEROSURVEY OF VIRAL HEPATITIS MARKERS IN DJIBOUTI.  
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Program, University of Maryland School of Medicine and Ministry of Public  
Health, Djibouti.

Djibouti has been lacking epidemiologic data on viral hepatitis. In October 1987, the first serosurvey was carried out which involved 656 individuals from various population groups: hospitalized patients, healthy city dwellers and healthy villagers. Each study subject had a medical interview and clinical examination performed. Sera were tested for hepatitis markers by commercial ELISAs. Hepatitis A infection was virtually universal in Djibouti (98.5% IgG seropositivity), and more than half of the individuals investigated had serum markers of hepatitis B infection. The rate of chronic HBsAg carriers was 7.3% and was higher for males (12.0%) than for females (5.2%;  $P < 0.01$ ). Both HBsAg and anti-HBs positivity rates were directly related to increasing age. Eleven sera tested positive for antibodies to the delta agent, all in healthy subjects testing also positive for HBsAg. There was a striking difference in the prevalence of delta seropositivity according to sex and ethnic groups, the highest rates were in males from the Afar ethnic group (7.7%), a rate 10 times higher than the delta infection rate in the combined rest of the study population (0.7%;  $P < 0.001$ ). No uniform mechanism could be found to account for transmission of HBV and the delta agent, and no correlations were noted between HBV-marker status and sexual promiscuity or the classic blood exposure risks. However, a significant association existed between the abuse of the drug Khat and the chronic HBsAg carrier state. (Supported by NMRDC, Bethesda, MD, Work Unit No. 3M161102BS10.AK.311)

- 75 PREPARATION OF A PURIFIED AND INACTIVATED HEPATITIS A VIRUS VACCINE.  
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Walter Reed Army Institute of Research, Washington, D.C. and Electro-  
Nucleonics, Silver Spring, MD

Human vaccination for hepatitis A virus (HAV) has been successfully demonstrated using an inactivated vaccine (HMI75/FI-1). Analysis of this unpurified vaccine indicated that only 0.17% of the total vaccine protein was HAV antigen; however this was adequate, in multiple doses, to stimulate the production of high levels of neutralizing antibodies to HAV. The majority of HAV antigen was found to be complete virions which could be separated from subviral antigen and host protein by continuous-flow zonal centrifugation in Renografin 76 (diatrizoate meglumine and diatrizoate sodium) gradients. The fractions containing complete virions were 99.8% free of host protein and appeared highly pure by analytical gel electrophoresis and electron microscopy. The potency of the purified HAV immunogen was compared to an unpurified HAV vaccine (FI-1, lot 2) by mouse inoculation. A single dose of 48 ng of the pure HAV seroconverted 50% of mice while 26.5  $\mu$ g was required for the unpurified vaccine. The mouse ED<sub>50</sub> dose could be reduced to <5 ng with the addition of alum adjuvant to the inoculum. These results indicate that continuous-flow zonal centrifugation is a practical and efficient technique for the production of pure and potent inactivated HAV vaccines.

ASTMH POSTER I

76 INACTIVATED HEPATITIS A VACCINE: FOLLOW-UP AND EVALUATION OF DIFFERENT SCHEDULES.

M.H. Sjogren, C.H. Hoke, L.N. Binn, K.H. Eckels, D.H. Dubois, L. Lyde, S. Oaks, R. Marchwicki, R. Lewis, W. Lednar, A. Tsuchida, K. Shafer, R. Chloupek, J. Ticehurst, D.S. Burke, and W.H. Bancroft. Walter Reed Army Institute of Research, Washington, D.C., USAMMDA, Frederick, MD, and Ft. Lewis, WA.

A formalin inactivated hepatitis A vaccine (HM175/FI-1) containing 17 ng/ml of hepatitis A virus antigen, was initially administered to 8 healthy men at 0, 1, 2, and 6 months with minimal side effects and stimulation of neutralizing antibody, detectable at 18 months following initial immunization, in all 8.

To increase our knowledge of vaccine safety and immunogenicity and test two schedules, we randomized 45 healthy men to receive vaccine in either a 0, 1, and 6 month or 0, 1, 2 and 6 month schedule. One ml was given intramuscularly in the deltoid area. Side effects, serum AST and ALT levels and signs of hepatitis were monitored. Potency of the vaccine was confirmed following administration by radioimmunoassay.

In the first 6 months, 115 doses have been given, and 101 questionnaires received. No evidence of hepatitis A was observed. Sore arms were reported by 44% (51% of these reported mild discomfort and 70% stated the soreness lasted less than 20 minutes), muscle aches by 22%, nausea by 3%, headache by 4%, and rash by none. Evaluation of the immune response is in progress. We conclude that this vaccine appears free of infectious virus, causes minimal side effects, and is safe for further testing.

77 MAPPING OF FUNCTIONAL DOMAINS OF THE DENGUE-2 VIRUS NONSTRUCTURAL GLYCOPROTEIN NS1.

J.R. Putnak. Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, D.C.

It has recently been shown by others that the NS1 glycoprotein exists predominately as a dimer in flavivirus infected cells. This dimer appears to be quite stable, but can be dissociated by boiling in SDS or by low pH which suggests that it is held together by noncovalent bonds. In order to study NS1 dimer formation, the gene coding for this protein was expressed in *E. coli*. Constructs containing as few as 175 a.a. of the N-terminus of NS1 were able to form dimers as well as trimers; whereas, the C-terminal 200 a.a. showed no tendency to oligomerize. These findings implicate N-terminal regions of NS1 in oligomer formation. However, N-terminal constructs of 240 a.a. or longer formed only dimers, not trimers. This result suggests that information contained in C-terminal regions of NS1 regulates dimerization or stabilizes the dimer state at the expense of higher-ordered polymers. Additional studies, carried out on dengue virus infected cells, showed that NS1 dimer formation, which was more efficient than that observed in *E. coli*, occurred before the protein exited the E.R. and did not require N-glycosylation.

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78 ANTIGENIC PROPERTIES OF VACCINIA VIRUS RELEVANT TO THE USE OF RECOMBINANT VACCINES.

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To complement studies of viral subunit immunogens that might ultimately serve as human vaccines, we analyzed antigenic properties of vaccinia virus, the live attenuated vector for several promising recombinant vaccines. We used both polyclonal and monoclonal antibodies (MAbs) to investigate epitopes relevant to vaccinia virus immunity. MAbs to a 28 Kd protein demonstrated the greatest biological activity: they reduced by more than 99% the number of vaccinia virus plaques on Vero cells at MAb concentrations as low as 1 µg/ml, and they exhibited significant protective efficacy against otherwise lethal vaccinia virus infections in mice. MAbs to a 34 Kd protein neutralized vaccinia virus by 50 to 90% but did not prevent or delay fatal infection. Non-neutralizing MAbs, including those against the 84 Kd (hemagglutinin) protein, also appeared to be ineffectual in vivo. Competition between unlabeled and biotinylated MAbs, as measured by ELISA, showed that a single topological site on the 28 Kd protein and three linked sites on the 34 Kd protein were associated with neutralization; multiple discrete topologic sites, including three on the hemagglutinin, were bound by non-neutralizing MAbs. More than 50 MAbs reactive with only seven vaccinia virus proteins were characterized initially. Since vaccinia encodes more than 100 polypeptides and hyperimmune mouse serum was found to react with at least 37 different proteins, properties of the initial battery of MAbs are being compared to those of polyclonal anti-vaccinia reagents in order to narrow the search for additional epitopes important in vaccinia virus immunity.

79 SURVEY OF HUMANS WITH UNDIFFERENTIATED ACUTE FEBRILE ILLNESS FOR RIFT VALLEY FEVER ANTIBODIES, EGYPT, 1985-87,

A.M. Zaki,<sup>1</sup> T.M. Ksiazek,<sup>2</sup> D.M. Watts,<sup>3</sup> M.A. Darwish,<sup>1</sup> and C.J. Peters,<sup>2</sup> <sup>1</sup>Ain Shams University, Research and Training Center for Vector-Borne Diseases, Cairo, Egypt, <sup>2</sup>U.S. Army Medical Research Institute for Infectious Diseases, Fort Detrick, Frederick, MD, and <sup>3</sup>U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

Rift Valley fever virus (RVFV) has not been detected in Egypt since the epidemic of 1977, but reintroduction or possibly even endemic activity continues to pose a threat to man and domestic animals. During 1985-87 acute sera were collected from patients with fever, malaise, and myalgia. IgG ELISA and neutralization tests detected RVFV-specific antibodies in 21/114 (18%), and 6 of the 21 (29%) also were positive by IgM capture ELISA. The IgM positive sera had definite reactivity (OD >0.2, titers >800), but less than observed in the wake of the 1987 epidemic in Mauritania (OD >1.0, titers >10<sup>7</sup>). High-titered Sandfly fever (SF) Sicilian and Naples antibodies were common in these sera and 3 had low-level IgM reactivity against SF. Rheumatoid factor could not be detected by latex agglutination or ELISA. None of the 4 patients for whom paired sera were available seroconverted, nor were epidemiological variables revealing. We conclude that these IgM antibodies reactive with RVFV deserve further study, but probably represent cross-reactions with another phlebovirus.

ASTMH POSTER I

80

VIRAL INFECTIONS IN BATS OF GUATEMALA.

Sonya R. Ubico and Robert G. McLean.\* Centers for Disease Control, Fort Collins, Colorado.

Neotropical bats were collected from different life zones in Guatemala in 1983-1984 to determine the presence and distribution of rabies virus and a variety of arboviruses and bat viruses. Rabies antigen was detected in brain smears by the fluorescent antibody test in 11 of 34 species of bats examined. Neutralizing (N) antibody against Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis, St. Louis encephalitis, vesicular stomatitis, Tacaribe and Rio Bravo viruses were detected in some resident species of bats. No N antibody against Nepuyo virus was detected. The geographic distribution of the different bat species and of the disease agents are presented.

81 AEROSOL INFECTION OF RHESUS MONKEYS WITH JUNIN VIRUS.

R.H. Kenyon, K.T. McKee, Jr., P. Zack, C. Crabbs, C. York, A. Frank, and C.J. Peters. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701.

Rhesus monkeys inoculated intramuscularly (IM) with human-virulent strains of Junin virus (Argentine hemorrhagic fever) develop a clinical syndrome and pathological lesions that closely mimic the disease in man. Since the natural route of human infection is believed to be by the respiratory route, we determined the disease course in monkeys subjected to small-particle aerosol infection. Two groups of 3 monkeys each received calculated inhaled doses of  $10^4$  PFU or  $10^2$  PFU of strain 3790. All monkeys became acutely ill in the third week after exposure. Some died in this acute phase, while others became chronically ill and were killed when they were cachectic. Disease courses of those animals exposed to the different doses were similar. All became viremic by 7 or 10 days postexposure. Oropharyngeal secretions contained high viral titers by day 10, and they usually remained positive until death. Virus was detected primarily in the visceral organs of those animals dying by the fourth week, while in those dying later virus was found primarily in the CNS. Alpha interferon (IFN) was detectable by day 3 in the monkeys receiving a high viral dose, and by day 7 in the low-dose group. Serum IFN levels peaked ( $10^4$  U/ml) in all monkeys at 10 to 14 days. In some monkeys IFN levels returned to baseline by day 17 but in others, levels could be measured up to the time of death. Gross and microscopic lesions as previously described for IM-infected monkeys were found in the spleen, lymph nodes, skin, and oral cavity in those examined in the third week of infection. Lesions were primarily confined to the CNS in the two monkeys killed on day 42. Monkeys infected with JV by an aerosol route developed a disease pattern indistinguishable from those infected IM.

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- 82 EVIDENCE FOR THE SPECIFIC BINDING OF RIFT VALLEY FEVER VIRUS TO COMPONENTS OF SOLUBILIZED MOSQUITO TISSUES AND CULTURED CELLS.  
A.A. Mikhail, M.G. Lozykowski, and W.S. Romoser, Ohio U., Athens, OH.

In an effort to isolate possible cell surface receptor molecules for Rift Valley fever (RVF) virus we have developed an enzyme-linked immunosorbent assay (ELISA) to test for the presence of specific virus-binding molecules in extracts of mosquito tissue.

Microtiter plates were coated with cell membrane preparations from homogenized whole adult mosquitoes or cultured *Aedes albopictus* cells (C6/36) and incubated overnight. After conventional blocking, RVF vaccine (formalin-killed virus) diluted with PBS was applied and incubated an additional 12 hours. The plates were washed vigorously and the presence of bound virions was detected by the addition of a mixture of monoclonal antibodies directed against RVF virus envelope glycoproteins, G<sub>1</sub> and G<sub>2</sub>, and against nucleocapsid protein. Subsequently, a conjugate of HRP and goat anti-mouse antibody was added. After reaction with substrate (o-phenylenediamine), optical density was determined with an ELISA reader.

Using the above assay we have found evidence of specific binding of RVF virus to a component or components of both whole body and cultured cell homogenates. Further, this binding is diminished upon treatment with 2-mercaptoethanol. We view this specific binding as a possible indication of the presence of mosquito cell surface receptor molecules for RVF virus.

- 83 PROTEINURIA IN MONKEYS INFECTED WITH PROSPECT HILL VIRUS.  
D.M. Asher,\* A. Collier, A.V. Wolff, R. Yanagihara, L.J. Murphy, K.L. Pomeroy, Z. Eldadah, C.J. Gibbs, Jr., and D.C. Gajdusek. National Institutes of Health, Bethesda, MD.

Primates were previously reported to be susceptible to infection with two hantaviruses, Hällnäs (nephropathia epidemica) and Prospect Hill, which produced mild proteinuria and equivocal abnormalities in liver function studies (Arch. Virol., in press). To confirm and extend those investigations, two healthy female cynomolgus monkeys were inoculated intravenously with 10<sup>5</sup> plaque-forming units of the original strain of Prospect Hill virus. Hematological studies, clinical chemistries, and renal clearances were performed at intervals before and after inoculation. Neither monkey showed any clinical sign of illness. Hematological and clinical chemistries, including blood urea nitrogen, serum creatinine and liver function studies, were unchanged. Clearances of endogenous creatinine and of phensulfonphtalein did not decrease. Modest increases in urinary total protein and albumin were detected in one monkey at the end of the third week after inoculation and for several days thereafter. In the other monkey increases in total urine protein and albumin were found at the end of the second week after inoculation, remaining intermittently elevated for more than two months. Urinary IgG levels were not increased in either animal. Although tests of glomerular filtration were not abnormal, the presence of significant proteinuria suggests an impairment of glomerular vascular integrity.

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- 84 **INACTIVATED VENEZUELAN EQUINE ENCEPHALOMYELITIS VACCINE: IMMUNOGENICITY OF AND REACTIONS TO A NEW LOT GIVEN AS A BOOSTER.** F. J. Malinoski\*, N. A. Popovic, J. A. Mangiafico, and G. F. Meadors III. Medical and Disease Assessment Divisions, USAMRIID, Ft. Detrick, Frederick, MD, USA

Venezuelan equine encephalomyelitis (VEE) is a mosquito-borne, viral disease that can cause significant human disease. A live, attenuated vaccine, TC-83 (derived from the Trinidad donkey strain), and several lots of a formalin-inactivated preparation of TC-83 (designated C-84) have been shown to be safe and effective in over 25 years of use at USAMRIID. We have analyzed the immune response and clinical reactions to a new lot of the inactivated vaccine (C-84-6) in 57 at-risk subjects who had previously received the live, attenuated vaccine. Increases in plaque-reduction neutralizing antibody titer (PRNT<sub>50</sub>) were observed in 89% of individuals. Of subjects examined for PRNT antibody to antigenically distinct epizootic subtypes, 70% had increased titers to group I-C and I-E subtypes. Five adverse reactions were noted. One systemic urticarial reaction occurred 4 1/2 hours after inoculation. Four other subjects had mild local or nonspecific systemic symptoms. All recovered without sequelae. The antibody response to this inactivated vaccine is similar to that found with other lots. The rate of significant, potentially serious adverse reactions is calculated as less than 2%. The finding of increased titers to group I-C and I-E subtypes suggests that booster immunization may broaden the initial protection induced by the live vaccine.

- 85 **GENETIC REASSORTMENT OF RIFT VALLEY FEVER VIRUS STRAINS IN CELL CULTURE AND IN THE MOSQUITO, *CULEX PIPPIENS***

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Following the observation that Rift Valley Fever virus (RVFV) was capable of high frequency reassortment in multiply-infected mammalian cell cultures, the ability of infected mosquitoes to generate reassortants was also investigated. Adult, female *Culex pipiens* were inoculated intrathoracically with both Egyptian (ZH501) and Senegalese (38661) RVFV strains, and the antigenic phenotypes of the progeny virus from individual mosquitoes were monitored at various times after infection. Individual plaques were selected, amplified in vero cells, and examined with monoclonal antibodies directed at an M segment marker (glycoprotein G1) and an S segment marker (nucleocapsid protein) which were capable of distinguishing the parental strains. Reassortants between the parental strains were detected at each time point tested (between 20 and 96 hours postinfection), and individual mosquitoes were found that contained both parental as well as various permutations of reassortant viruses. Reassortants were inoculated into mosquitoes, and were found to replicate efficiently and be transmitted to hamsters. The use of reassortants provides a means to examine the contributions of individual genome segments to viral infectivity, pathogenicity, and virulence, and has been utilized successfully to localize attenuating mutations in a live-attenuated RVFV vaccine strain. The ability of RVFV to form genetic reassortants may play a significant role in the evolution of virus strains in nature.

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THE GEOMETRIC MEAN AND ITS USE FOR EGG COUNT DATA  
IN SCHISTOSOMIASIS.

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The use of the arithmetic and geometric means as measures of central tendency for egg excretion data in schistosomiasis is discussed. The geometric mean is considered the more appropriate summary statistic, but is only valid for position counts. The  $(\ln(x+1))$  transformation, employed to circumvent the constraint that all counts must be positive, is discussed and rejected. Assuming that egg count data are distributed log-normally, the geometric mean is not unbiased, but dependent upon sample size and skewness. High geometric mean egg counts may only be a function of sample size. The log-normal distribution and its usefulness for estimating the proportion of the infected population at increasing egg output levels are briefly discussed. Data published by Uemura are used as an illustrative example.

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PREVALENCE OF ANTI-DELTA IN HBsAg(+) PATIENTS WITH CIRRHOSIS AND  
BLOOD DONORS IN CENTRAL TUNISIA.

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Hepatitis B virus infection and cirrhosis are associated in Central Tunisia (Ann Trop Med Parasit 1983;77:223) but prevalences of delta infection in such patients and in blood donors were unknown. To investigate this point, patients with cirrhosis diagnosed by laparoscopy and/or liver biopsy (33 cases) and blood donors were recruited in Sousse University Hospital from 1985 to 1987. Sera were tested for HBsAg by radioimmunoassay (RIA); HBsAg(+) sera were tested for anti-delta by RIA (Abbott Lab.). 26 HBsAg(+) cirrhosis patients (M:F sex ratio: 2.25, age range: 24-80) and 42 HBsAg(+) blood donors (sex ratio: 5.00, age range: 14-39) were identified. Among the HBsAg(+) subjects, the prevalence of anti-delta was 61.5% (16/26) in cirrhosis patients and 28.6% (12/42) in blood donors ( $X^2=7.22$ ,  $p<0.01$ ). Although cirrhosis and blood donors differed in terms of age and sex, these results do not exclude the existence of a relationship between delta agent and cirrhosis.

## ASTMH POSTER I

### CAMPYLOBACTER ENTERITIS AMONG AIDS PATIENTS IN LOS ANGELES COUNTY

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Although surveys have shown that Campylobacter is the most commonly isolated agent from patients with acute diarrhea in the United States, little data exists regarding the relative importance of Campylobacter enteritis among patients with AIDS in part because Campylobacter infections are not reportable in many localities. In Los Angeles County Campylobacter was made reportable in 1983 and a surveillance system established. To determine the incidence of Campylobacter among AIDS patients, surveillance data for Campylobacter was matched to the AIDS reporting system registry for the time period January 1983 through April 1988. Sixty-four (1.3%) of 4916 AIDS cases were reported to have Campylobacter infection (38 C. jejuni, 2 C. fetus, 1 C. cinaedi, 23 species unknown). By comparison, Cryptosporidium was found in 3.9% and Salmonella in an estimated 2.0% of AIDS patients. The age-adjusted rate of Campylobacter among AIDS cases, 1002/100,000, was 14 times the crude population rate of 69.6/100,000 over the same time period. A distinct seasonal variation was noted with peaks occurring in both June (12 cases) and November (10 cases). In approximately 30% (22) of these patients Campylobacter was diagnosed concurrent with, or within 2 months of, their diagnosis of AIDS. Campylobacter infection was significantly more common among female AIDS patients ( $p < .01$ , OR=3.6 95% CI 1.36,8.77) and foreign born cases ( $p = .04$ , OR=2.1 95% CI 1.04,3.96). No age- and race- specific differences were observed and no association with other underlying AIDS related conditions were noted. Overall mortality among AIDS patients with Campylobacter (78%) was greater than those patients without Campylobacter (62%). ( $p < .02$ , OR=2.1 95% CI 1.14,4.04) Of 41 cases for whom data was available 22 (54%) were hospitalized with their infection. Eleven patients (27%) were affected by protracted diarrhea (>2 weeks duration). Campylobacter enteritis occurs commonly in AIDS patients in Los Angeles County and infection may predispose to a more fulminant AIDS course. The apparent gender disparity and association with foreign-born cases of Campylobacter infection in AIDS patients warrant further study.

- 89 EVALUATION OF MAGAININ ACTIVITY AGAINST PROTOZOAN PATHOGENS. G. L. McLaughlin\*, J. E. Stimac, and C. E. Kirkpatrick. University of Illinois.

Magainins are 23-amino acid long peptides derived from the skin of Xenopus laevis which were first described by M. Zasloff (1987 PNAS 84: 5449-53; 1988 PNAS 85: 910-3) as having broadspectrum antibacterial and antiprotozoal activity. In order to screen additional pathogens for sensitivity to magainins, we synthesized three peptides: magainin 1 (GIGKFLHSAGKFGKAFVGEIMKS); magainin 2 (K and N at positions 10 and 23); and rammag 2, a control peptide with the amino acids of magainin 2 in a random sequence. Lyophilized powders contained approximately 50% by weight of full-length peptides as determined by amino acid analysis. Peptides were added to liquid media at concentrations from 1 to 500 ug/ml, to cultures of: Escherichia coli strain HB101; a drug-resistant Pseudomonas aeruginosa isolate from an eye infection; corneal epithelial cells; pathogenic and nonpathogenic Acanthamoeba species; several Leishmania species. Cell morphologies and numbers were scored at 1 hr, 3 hrs, and after overnight growth. The order of sensitivity to magainins was: amoeba, bacteria, Leishmania, and corneal cells. For particular cells and magainin concentrations, specific effects included cell lysis, decreased mobility, rounding, and growth inhibition; magainin effects were partially reversible with cell washes. Although for many diseases, practical considerations such as delivery routes may limit their utility, magainins do display significant activity against a wide range of pathogens. Supported in part by PHS grant EY07651 from the National Eye Institute and by AI24865 from NIAID.

ASTMH POSTER I

- 90 TUMOR NECROSIS FACTOR IN AEROMONAS-INFECTED RABBITS. N.D. Pacheco\*, D.H. Burr, J. Eckstein and F.M. Rollwagen. Naval Medical Research Institute, Bethesda, MD.

Tumor necrosis factor (TNF), an important primary mediator of the inflammatory response, was assayed in serum of rabbits infected with clinical isolates of Aeromonas spp. Cytotoxicity of L929 cells in the presence of actinomycin D was used to estimate TNF levels. One rabbit received nonvirulent Aeromonas injected intraintraintestinally, did not develop diarrhea, and had TNF levels at 12 hr that were in the normal range. Another rabbit receiving the same strain developed diarrhea and had TNF levels 4 times normal. Neither rabbit died during the 5-day experiment nor was septic at 12 hr or 5 days. An additional two rabbits were bled at 10 hr, immediately before death. These had received virulent strains intraintraintestinally and had TNF levels 23 times normal. Neither rabbit developed diarrhea but both were bacteremic. One rabbit receiving a virulent strain intraperitoneally did not develop diarrhea and was not bacteremic at 12 hr or 5 days but had TNF levels 13 times normal at 12 hr and 34 times normal at 5 days.

The induction of endotoxic shock by TNF could contribute to death following infection with Aeromonas. In those animals in which an hepatic dissemination of organisms occurred, neutrophil (heterophil) infiltration surrounding the areas of bacterial colonization was observed. Since TNF can augment the activity of neutrophils, this may provide an important role for this mediator in clearance of bacterial infections. The role of TNF in infection can be either beneficial, such as in its neutrophil activation, or detrimental, as in its ability to mediate endotoxic shock. From this perspective, a balance of beneficial and detrimental activities is desirable.

- 91 CONTINUOUS CULTIVATION OF BARTONELLA BACILLIFORMIS  
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Bartonella bacilliformis is a hemotrophic bacteria responsible for a biphasic disease causing significant morbidity and mortality in Peru's inter-andean valleys: Oroya fever with a hemolytic macrocytic anemia and high mortality. "Verruga Peruana", with bacterial invasion of the capillary endothelial cells leading to proliferation and formation of hemangioma-like papules with low mortality. A novel technique for rapid isolation and continuous cultivation of Bartonella bacilliformis from blood samples in the acute febrile illness has been achieved using the method for P. falciparum cultivation. Infected washed red blood cells were resuspended in RPMI-1640 supplemented with Hepes buffer (25 mM) and 0.2% NaHCO<sub>3</sub> with 15% fetal bovine serum. Uninfected washed human erythrocytes were added to make a 8% suspension, dispensed in tissue culture flasks and incubated at 28°C in a candle jar. Subcultures were performed weekly for a period of four months. Intracellular bacilli and free pleomorphic organisms were observed in Giemsa stained thin blood smears. Bartonella bacilliformis has been isolated from 22 blood samples in the second phase of the disease "Verruga Peruana" from 3 biopsies and 10 aspirates from the miliar and nodular forms. Identification of the isolates were on basis of morphology growth and biochemical characteristics. The described systems for cultivation of Bartonella bacilliformis will be useful in determining entry mechanisms of this organisms into erythrocytes, biochemical and immunological studies.

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92 ETIOLOGY OF FEVERS OF UNKNOWN ORIGIN IN PATIENTS ADMITTED TO THE MILITARY HOSPITAL, RAWALPINDI, PAKISTAN.

R.E. KRIEG\*, J.F. DUNCAN, J.P. BRYAN, B. AWAN, A. AHMED, M. RIAZ, S. NABI, J.E. SIPPEL, and N. BUKHTIARI, USUHS and NMRI, Bethesda, MD and PULSE, AMC, Rawalpindi, Pakistan.

Adult males admitted to the Military Hospital with acute febrile illness, negative chest x-ray, and no meningeal signs were studied to determine the etiology. One blood, fecal and urine specimen per patient was cultured. The sera were tested with the Widal test and Salmonella and Brucella IgG and IgM antibody ELISAs. The KPL ELISA screening kit for Salmonella was used for antigen detection in serum and enrichment cultures. Of the 241 febrile patients identified from October 1986 to December 1987, malaria was diagnosed in 37 and enteric fever in 120. Of these 120 patients, Salmonella was isolated from 84 (70%); S. typhi in 61, S. paratyphi A in 20, S. spp. in 3. Blood cultures were positive in 56 (67%) while Salmonella was isolated only from stool in 26 (31%) and by string capsule in 2. There were 3 S. typhi, 2 S. paratyphi A, and 2 S. spp. resistant to AMP, CHLORO, and TMP/SXT. In the stool culture positive cases, antigen was detectable in all the enrichment cultures following overnight incubation. Anti-Salmonella IgG was present in 80% of culture positive patients and 45 (54%) had an initial Widal titer  $\geq 1:160$ . Serologically, one patient had acute brucellosis. A single blood and stool culture from patients in an area of high endemicity for typhoid fever is diagnostic in 70% of patients clinically suspected of having enteric fever. The ELISA for IgG antibody had the highest positive predictive value (76%).

93 TUBERCULIN SKIN TEST CONVERSION RATES IN ACTIVE DUTY NAVAL AND MARINE CORPS PERSONNEL, 1980-1986

E.R. Cross,\* R. Wallace and K.C. Hyams, Naval Medical Research Institute, Bethesda, MD, and Naval Medical Data Services Center, Bethesda, MD

The Navy Medical Command (NMC) instructs all personnel to be screened for tuberculosis by the Mantoux method every 3 years with personnel at high risk to be screened periodically. A new reactor is defined as an individual who has a 5 TU tuberculin skin test reaction with an area of induration measuring 10 mm or greater within 48 to 72 hours. Between 1980 and 1986, a total of 3,029,874 tuberculin tests were read in Naval outpatient facilities and in sick bays aboard ships. Of these, 0.8% converted. This is well within in the NMC expected conversion rate of 1 to 2%. However, higher conversion rates were observed in different locations. For example, the conversion rate in the Naval outpatient clinic in the Philippines was 3.1%, in Japan, 2.5% and in Korea, 2.0%. In the United States, outpatient facilities in northern California had conversion rates of 1.6%, in Washington State, 1.5% and in Cuba and Puerto Rico the rate was 1.4%. Although the overall shipboard rate was 0.8%, ships in the Pacific area had a conversion rate of 1.0%, almost double the 0.6% rate for ships in the Atlantic area. Auxiliary ships (A) and Carriers (C) had the highest conversion rates (0.9%) with Attack Aircraft Carriers (CVA) having a rate of 1.6%.

ASTMH POSTER I

- 94 **CAMPYLOBACTER INFECTION AMONG INFANTS AND CHILDREN, SANAA, YEMEN ARAB REPUBLIC.** R.L. HABERBERGER, JR.<sup>1</sup>, M. HUSSEIN<sup>2</sup>, M. ISHAK<sup>3</sup>, AND J.N. WOODY<sup>1</sup>. U.S. NAVAL MEDICAL RESEARCH UNIT NO. 3, CAIRO, EGYPT<sup>1</sup>, MINISTRY OF HEALTH, CENTRAL PUBLIC HEALTH LABORATORY, SANAA, YEMEN ARAB REPUBLIC<sup>2</sup>, AND REVOLUTION HOSPITAL, SANAA, YEMEN ARAB REPUBLIC<sup>3</sup>.

We report on *Campylobacter* infection among infants and children in a point-prevalence, case-control survey during February 1988. This work was undertaken to determine: (a) the point-prevalence of etiologic agents of severe diarrheal illness among children; (b) antibiograms of enteropathogens isolated in Sanaa, Yemen Arab Republic; (c) risk factors associated with the etiology of diarrheal diseases; and (d) strategies for preventing or interrupting disease transmission of the various etiologic agents. We examined 122 (63 cases, 59 controls) neonates and children to 14 years of age. Twenty-four percent of the cases and 30% of the controls were one year of age or under. Of the cases, *Giardia* was identified in 44%, *Salmonella* Group B (24%), Rotavirus antigen (13%), *Shigella* Group A (8%), *Shigella* Group B (8%), *Campylobacter* spp. (5%), *Clostridium difficile* toxin (5%), *Shigella* Group C (2%), and *Shigella* Group D (2%). Controls were colonized with the following organisms: Rotavirus antigen (34%), *Campylobacter* spp. (30%), *Giardia* (5%), *Shigella* Group B (12%), *Shigella* Group C (6%), *Shigella* Group D (2%) and Adenovirus antigen (2%). Dual infections were detected in 4 of 63 cases (6%) versus 13 of 59 controls (22%). Significant differences were seen in the antibiograms of isolates from cases versus controls, with the case isolates demonstrating increased resistance to multiple antimicrobial agents. The newer quinolones (cinoxacin, norfloxacin) appear to be the only group of antimicrobial agents we tested to which all our bacterial isolates were universally susceptible *in vitro*. Supported by NMRDC Work Unit No. 3M161102BS10.AK311.

- 95 **FIRST CASE REPORT OF INTESTINAL CAPILLARIASIS IN EGYPT.**  
N.S. Mansour\*, F.G. Youssef, and E.M. Mikhail  
U.S. Naval Medical Research Unit No.3, (NAMRU-3).

*Capillaria philippinensis* was reported from Philippines, Thailand, Japan and Iran. In the present communication, we report on the first case of intestinal capillariasis in Egypt. A 41-year old female physician from Cairo suffered from severe diarrhea, vomiting, gurgling stomach, abdominal pain and distension, malaise, edema, and anorexia for more than 3 months during which she lost 20 Kgm of her body weight. The blood picture and the liver function tests were normal except for low protein level. The barium meal X-ray showed thickening of the jejunal wall with some constrictions. Intestinal biopsy showed exudate of plasma cells and lymphocytes in the stroma. Ultrasonography did not show any masses or ascites or any abnormalities in the liver, gall bladder, pancreas and the intestines. Repeated stool examination revealed few larvae and large number of adult worms and eggs of *Capillaria philippinensis*, Charcot Leyden crystals, abundant fat droplets and undigested food particles. Treatment with 200 mg of Flubendazole (R17889) twice daily for 30 days resulted in complete clinical and parasitological cure. Since this patient never travelled abroad but frequently prepared and ate local and imported fish, the various possible means of contracting infection will be discussed in this presentation.  
(Supported by NMRDC, Bethesda, MD, Work Unit No. 3M161102BS10.AK.311).

ASTMH POSTER I

96 METRIFONATE OR PRAZIQUANTEL TREATMENT IMPROVES PHYSICAL FITNESS AND APPETITE OF KENYAN SCHOOL BOYS WITH SCHISTOSOMA HAEMATOBIIUM AND HOOKWORM INFECTIONS.

L.S. Stephenson,\* M.C. Latham, K.M. Kurz, and S.N. Kinoti. Division of Nutritional Sciences, Cornell University, Ithaca, NY and Medical Research Center, a Department of KEMRI, Nairobi, Kenya.

We studied physical fitness with the Harvard Step Test (HST) and ad lib intake of a morning snack (maize meal porridge) in primary school boys infected with S. haematobium (100% baseline prevalence) and hookworm (94-100% prevalence) who received a single dose of metrifonate (MT, 10 mg/kg body wt) or praziquantel (PR, 40 mg/kg body wt) or a placebo (PL). Boys were examined (Exam 1), allocated to MT, PR or PL groups (n=16 per group), treated, and re-examined 5 wk later. The groups did not differ significantly at Exam 1 in age, anthropometry, hemoglobin level, prevalence or intensity of S. haematobium and hookworm, or in initial HST scores and porridge intake. After treatment, the MT and PR groups improved significantly in HST score and porridge intake but did not differ significantly from each other, while the PL group did not change. From Exam 1 to 2, the MT group showed a significant decrease in the logs of S. haematobium egg counts ( $\bar{X}$  180 vs 14 e/10 ml adj,  $p < .0002$ , 82% egg reduction) as did PR ( $\bar{X}$  198 vs 0.1 e/10 ml adj,  $p < .0002$ , 99.9% reduction) while the PL's did not change. The MT group also exhibited a significant decrease in log of hookworm egg count ( $\bar{X}$  1,550 vs 75 epg,  $p < .0005$ , 80% reduction). We conclude that treatment with either drug may allow improved physical fitness and appetite in areas such as this one where S. haematobium, hookworm and protein-energy malnutrition are highly prevalent. Supported in part by Edna McConnell Clark Foundation grant 284-0120.

97 THE TREATMENT OF ACUTE FASCIOLA HEPATICA INFECTION IN CHILDREN.

Z. Farid\*, N. Mansour, M. Kamal, K. Kamal, Y. Safwat, and J.N. Woody. U.S. Naval Medical Research Unit No 3, (NAMRU-3), and Abbassia Fever Hospital (AFH), Ministry of Health, Cairo, Egypt.

During the summer of 1987, 15 children, 10 girls and 5 boys aged 5 to 10 years, were referred to hospital for investigation of prolonged fever and eosinophilia. All were acutely ill, complained of abdominal pain and diarrhea, and had enlarged tender livers. The admission sera in every child reacted positively to purified Fasciola antigen by counterimmunoelectrophoresis and enzyme-linked immunosorbent assay (ELISA) tests. Fasciola hepatica eggs were eventually found in the stools of every child.

In acutely ill children, fever and toxemia were controlled with prednisone, 5 to 10 mg given daily before starting antiparasitic therapy. Three children were treated with intramuscular dehydroemetine, 1.0 mg/kg body weight, given daily for 14 doses and 12 children were treated with oral bithionol, 40 mg/kg body weight, given on alternate days for 14 doses. Eight children were cured. Three weeks after completing therapy, 7 children, 2 treated with dehydroemetine, and 5 with bithionol had a recurrence of fever and passed F. hepatica eggs in the stools. Four of these 7 children were successfully retreated with bithionol using a higher dose of 50 mg/kg given daily, instead of on alternate days, for 10 doses. There were no adverse side effects. We previously reported on the difficulty of treating this trematode in heavily infected children and we suggest that the use of daily oral bithionol may result in better cure rates. Further studies will be conducted in the summer of 1988. (Supported by NMRDC, Bethesda, M.D. Work Unit No. 3M464758D849.8H.341).

## HIGH-LEVEL GENTAMICIN RESISTANT ENTEROCOCCI FROM ZIMBABWE.

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New Haven, CT; University of Zimbabwe, Harare, Zimbabwe.

High-level gentamicin resistance (MIC  $\geq 2000$  mcg/ml) in enterococci eliminates bactericidal activity previously achieved with a penicillin or vancomycin and aminoglycoside combination. These resistant enterococci have been reported from several countries but have not yet been reported from Africa. Rectal swab specimens were collected from 28 patients on a medical ward in a referral hospital in Zimbabwe from February to March 1988 and screened for these resistant isolates. Risk factor data including age, previous antibiotic exposure, and duration of hospitalization were also collected. 8/28 (29%) of these specimens contained high-level gentamicin resistant enterococci. A case control study showed that previous exposure to beta-lactam antibiotics or aminoglycosides were significant risk factors ( $p < 0.05$ ) for colonization with these organisms. This study documents the presence of high-level gentamicin resistant enterococci in Africa, and perhaps reflects the selective pressure for gentamicin resistance caused by aminoglycoside usage. If resources permit, screening for high-level gentamicin resistance should be considered in enterococcal isolates causing serious infection when resistance has been demonstrated in a hospital setting.

## 99 SEROLOGICAL EVALUATION OF PATIENTS WITH EOSINOPHILIA AND HELMINTHIC INFECTIONS: A PRELIMINARY STUDY.

N.A. El Masry,\* H. Shaheen, S. Bassily, K. Kamal, Z. Farid, and  
A. Ghaly. U.S. Naval Medical Research Unit No.3, Cairo, Egypt.

Twenty nine patients with parasitic infection and with varying degrees of peripheral eosinophilia were studied for levels of immunoglobulins G & M, using the enzyme linked immunosorbent assay (ELISA). Parasitic infections included Schistosomiasis 26, *Fasciola* 3, *Ascaris* 2 and Hookworm 6 patients. The antigens used were prepared from *Schistosoma mansoni* adult worms (SWAP), eggs (SEA), *Fasciola gigantica* worms and *Trichinella spiralis* larvae. Patients were categorized as group I; patients with more than 30% eosinophilia, group II; 16-30% and group III; 6-15%. Arbitrarily, levels of IgG were tabulated as (+1) to (+4). Groups I & II showed 73% at (+4) IgG level while in the third group (6-15% eosinophilia) only 15% showed (+4) IgG level. SEA antigens detected higher IgM levels than IgG in 6 patients indicative of recent exposure to schistosomiasis. 14 patients were followed up for 2 to 8 weeks post antiparasitic treatment and all showed consistent elevation of eosinophilia parallel with increase in parasite - specific IgG levels. These preliminary data show that eosinophilia is positively correlated with the parasite - specific IgG levels. Further studies are aimed at expanding the size of the study population and the battery of parasitic antigens to provide definite correlation between immunoglobulin levels and eosinophilia.

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**NUTRITIONAL STUDIES ON CONGOLESE CHILDREN**

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The nutritional and fatty acid status of an unselected group of 111 children from the village Bouansa, People's Republic of Congo, was investigated. Results showed by anthropometrical classifications a high prevalence of moderate malnutrition. Low levels of plasma proteins and high levels for immunoglobulins and circulating immune complexes were found. Anthropometrical data did not correlate with plasma proteins. Children with C-reactive protein > 9mg/l (n=25) had low prealbumin values and increased levels of ferritin. In order to critically assess the anthropometrical and biochemical findings it is necessary to take into consideration the apparently healthy appearance of the investigated children, which indicates a certain degree of adaptation to the limited availability of food and a high rate of acute and chronic infections.

The fatty acid status of a subgroup of 84 Congolese children was assessed by determination of fatty acids of plasma phospholipid and cholesterolester fractions through capillary gas chromatography. In comparison to values from North American and European children a wide range of values and low mean levels of linoleic acid, arachidonic acid and dihomo-gamma-linoleic acid were found. On average n3-fatty acids were slightly higher, while no differences occurred in monoenoic and n9-fatty acids. The sum of trans fatty acids was approximately within the range of German infants. However, the relation between the different trans fatty acids differed. Cholesterolesters contained more trans fatty acids than phospholipids.

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**RESULTS OF 859 SONOGRAPHICAL EXAMINATIONS AT A REGIONAL HOSPITAL UNDER TROPICAL CONDITIONS**

D. Franke, E. Doehring-Schwerdtfeger, G. Mohamed-Ali, I.M. Abdel-Rahim, R. Kardorff, M. Dittrich, J.H.H. Ehrich, Kinderklinik Med. Hochschule, 3 Hannover 61 and 65 Mainz, FRG and University of Gezira, Wad Medani, Sudan

In order to improve diagnostic facilities an ultrasound service was established at a major hospital in Wad Medani, Sudan. During the implementation period from November 1986 to March 1987, cooperative examination by Sudanese and German doctors covered Internal Medicine (n=384), Obstetrics and Gynecology (n=288), Surgery (n=106), Pediatrics (n=67) and other specialties (n=14). The most frequent indications were hepatobiliary diseases (21 %), complications of pregnancy (21 %), urinary tract lesions (15 %) and palpable abdominal masses (12 %). The ratio of pathological to normal ultrasound reports was 3:1. Pathological findings involved the liver (39 %) and biliary system (15 %), consisting of diffuse (n=209), vascular (n=78) and focal (n=28) abnormalities, biliary stones (n=42) and characteristic features of hepatosplenic schistosomiasis (n=50). 34 % of all examined pregnancies (n=213) showed a variety of pathological conditions ranging from fetal problems (n=30), oligo- or polyhydramnios (n=15) to placental abnormalities (n=15). Urinary tract pathology mainly comprised of primary renal disease (n=47) and obstructive uropathy (n=30). Since April 1987 more than 2000 further examinations have been performed by Sudanese doctors during clinical routine. We conclude: ultrasonography represents a very useful tool of intermediate medical technology that can successfully be applied in tropical countries with high diagnostic efficacy.

ASTMH POSTER I

- 102 EFFECT OF DIETARY FIBER ON EXPERIMENTAL GIARDIASIS. G.J. Leitch, G.S. Visvesvara, S.P. Wahlquist and C.T. Harmon. Morehouse School of Medicine and Centers for Disease Control, Atlanta, GA.

Young adult male Mongolian gerbils were maintained for 2 or 8 weeks on a defined pelleted high fiber diet, or a defined pelleted low fiber diet. All feeding was ad libitum. The daily stool weight and the cecum to body weight ratio of animals on the low fiber diet were 35% and 65% respectively of the values measured in animals on the high fiber diets. When gerbils were administered 100 G. lamblia cysts, strain CDC:0284:1 per os, 79% and 100% of the animals on the low fiber diet for 2 and 8 weeks respectively were infected and exhibited signs of enteropooling (a statistically significant increase in small intestine: body weight ratio), while only 36% and 38% of animals on the high fiber diet were infected. Infected animals on the defined high fiber diet had lower numbers of duodenal trophozoites at the time of sacrifice (14 days post cyst inoculation) and passed fewer cysts when compared to infected animals on the low fiber diet. This reduced cyst passage was not due to the cysts being retained in the enlarged cecum as cecal cyst recovery was lower in the infected animals on the high fiber diet. The following factors are currently being examined in an attempt to explain the effect of high dietary fiber on experimental giardiasis: gastric emptying, small intestinal transit, gastric and duodenal pH, and duodenal mucus blanket acidic glycoprotein content. To date no significant differences have been found in any of these factors between animals on a high and a low fiber diet and therefore no one of these factors can be used to explain the observed effect of dietary fiber on giardiasis. (Supported in part by U.S.A.I.D. Grant No. DAM 5054-655-6031-00).

- 103 ANALYSIS OF ANTI-GIARDIA ANTIBODIES IN PATIENTS WITH GIARDIASIS. J.P. Nowakowski\*, D.R. Hill. Division of Infectious Diseases, University of Connecticut School of Medicine, Farmington, CT.

We examined the serologic response to Giardia lamblia in patients with symptomatic giardiasis of varying duration (7-468 days). An ELISA using disrupted G. lamblia trophozoites (WB strain) as antigen and horse radish peroxidase-conjugated antihuman immunoglobulin (anti-IgG, -IgM, -IgA) was performed on sera (diluted 1:8) obtained from 24 healthy controls (Group A), 21 patients with non-Giardia gastrointestinal disorders (Group B), and 29 patients (37 sera) with confirmed giardiasis (Group C). Groups A and B did not differ for any antibody classes. Group C differed from A and B when compared separately or combined: mean optical density for IgG in Group C was  $1.703 \pm .322$  (SD) vs.  $1.139 \pm .276$  for A and B; for IgM,  $0.693 \pm .319$  vs.  $0.467 \pm .190$ ; and for IgA,  $1.577 \pm .406$  vs.  $0.973 \pm .299$  (all  $P < .001$ ). Group C was divided into 2 groups based on the interval from symptom onset to serum sampling (Group 1C: <30 days, n=12; Group 2C: >30 days, n=20). No significant differences were found for IgG or IgA. IgM was significantly higher for Group 1C vs. 2C ( $0.849 \pm .368$  vs.  $0.577 \pm 0.250$ ,  $P < .02$ ), with only 7/20 (35%) in Group 2C having a (+) IgM. The sensitivity and specificity of a (+) IgM for detecting giardiasis in Group 1C was 66.6% and 93.3%, respectively. In Group 1C 5/12 treated before serum sampling had a lower IgM than those treated after serum sampling ( $0.618 \pm .134$  vs.  $1.015 \pm .367$ ,  $P = .061$ ), suggesting an effect of therapy on IgM development.

Anti-Giardia IgM may be useful in identifying patients with giardiasis. Although IgM levels decreased in most patients within 30 days of onset, they can remain elevated in a small percentage for several months. Anti-Giardia IgG and IgA can persist for months irregardless of the duration of symptoms.

ASTMH POSTER I

- 104 SENSITIVITY OF GIARDIA LAMBLIA TO PROTEIN SYNTHESIS INHIBITORS:  
CORRELATION WITH SEQUENCE AND STRUCTURE OF RIBOSOMAL RNA.  
T.D. Edlind. Medical College of Pennsylvania, Philadelphia, PA

The very limited development of anti-parasitic agents targeting protein synthesis stems in part from the belief that parasite and host ribosomes are sufficiently similar to preclude selective toxicity. However, we have shown that Giardia ribosomal RNA has an unusual size and sequence (Nuc. Acids Res. 15,7889-7901); consequently, this organism and its rRNA provide a useful model for the development of protein synthesis inhibitors with anti-parasitic activity. In this study, we determined the sequence and secondary structure of the 3' end of the small subunit (16S) RNA. This region is involved in mRNA binding and tRNA decoding, and is the site of aminoglycoside inhibitory activity. The primary structure of these 140 nucleotides includes two blocks of sequence highly conserved among other organisms; the presence of U-1495 within one of these blocks predicts hygromycin sensitivity. The remaining sequence, while not conserved, can be folded into a secondary structure common to other rRNAs. Interestingly, a specific base pair (C-1409/G-1491) implicated in paromomycin sensitivity is present; while all prokaryotes have this base pair, it is absent in most eukaryotes (including mammals). A growth inhibition assay was used to test the sensitivity of several Giardia strains to a variety of aminoglycosides. After 72 hours, seven of nine aminoglycosides tested failed to inhibit growth at up to 250  $\mu\text{g/ml}$ . Paromomycin and hygromycin, however inhibited growth 50% at 30-60  $\mu\text{g/ml}$  and 90% at 60-120  $\mu\text{g/ml}$ , depending on the strain. These results correlate well with the sequence and secondary structure analysis. Paromomycin may be clinically useful where the toxicity of standard anti-giardial drugs is of concern.

- 105 TVF: A SOLUBLE CELL-DETACHING FACTOR SECRETED BY  
TRICHOMONAS VAGINALIS.

W.B. Lushbaugh,\*A.C. Turner and P.C. Klykken. The University of Mississippi Medical Center, Jackson, MS.

*T. vaginalis* grown in Dulbecco's modified Eagle's medium, with or without serum, produced a factor (TVF) which altered the morphology of selected mammalian cells *in vitro*. TVF had a molecular weight between 100-200 Kd by gel filtration and SDS-PAGE and was heat (56°C, 30 min) and pH (6 < > 8) labile. Co-incubation of TVF with adherent target cells caused a marked rounding and clumping of BHK-21 or CHO-K1 cells, but had no effect on RK-13 or WEHI-3 cell lines. These morphologic changes were concentration, time, and energy dependent. Reversibility was attained by exogenous serum addition (>10%) or TVF washout. Target cell perturbations were not accompanied by significant changes in growth as measured by nuclei counts, DNA content and  $^3\text{H}$  thymidine incorporation, or in cell leakage as assessed by lactate dehydrogenase activity and trypan blue dye exclusion. TVF-induced effects were also independent of cyclic AMP and cyclic GMP levels in BHK cells exposed to TVF for 5 min to 24 hrs. Observed morphologic alterations mediated by TVF *in vitro* are consistent with the dysplastic hypercellularity seen in symptomatic *Trichomonas* vaginitis. This study was supported in part by grants from the NIH (BRSO SO7RRO5386) and the RJR/Nabisco Foundation.

ASTMH POSTER I

- 106 CLONING AND CHARACTERIZATION OF THE MAJOR ANTIGENS OF ENTAMOEBA HISTOLYTICA RECOGNIZED BY HUMAN IMMUNE SERA. E.L.W. Kittler, W.A. Petri Jr., and J.I. Ravdin. Univ. of Virginia School of Medicine, Charlottesville, VA.

In addition to the 170 Kd Gal/GalNAc adherence lectin, antibodies in sera of patients cured of invasive amebiasis (liver abscess or colitis) most frequently and prominently recognize E. histolytica (Eh) proteins of 37, 43, 59, 90, and 110 Kd on Western blots. Recurrence of invasive amebiasis is rare, suggesting that a humoral or cell mediated immune response to these major Eh antigens is protective. A pool of human immune sera (H.I.S.) was used to screen an Eh cDNA library of  $5.6 \times 10^5$  clones constructed from polyA-mRNA in the vector Lambda ZAP (Stratagene). The Lambda ZAP vector cloning site lies within the  $\beta$ -galactosidase gene, allowing expression of an inserted Eh cDNA as a  $\beta$ -gal fusion protein. Nonspecific reactivity of H.I.S. to E. coli protein was eliminated by passage through an affinity column consisting of total E. coli protein linked to Sepharose 4B. Ten individual cDNA clones were specifically recognized by H.I.S. on the first screen and are now being characterized. An Eh genomic library of  $6.3 \times 10^3$  individuals has been constructed. Hae3-Alu1 partial restriction digests of Eh genomic DNA were size-selected for 2.0-4.2 Kb range, ligated to EcoR1 adapters, and inserted into the multiple cloning site of Lambda ZAP. The cDNA clones which express antigenic fusion proteins corresponding to major Eh antigens will be used to recover antigen encoding genes from the Eh genomic DNA library. These genes will be sequenced and used to produce "full-length" antigenic Eh fusion proteins for determination of their efficacy as protective immunogens.

- 107 COMMON OCCURRENCE OF ASYMPTOMATIC CRYPTOSPORIDIUM AMONG INFANTS AND TODDLERS IN A NEW YORK CITY DAY CARE CENTER. F.G. Crawford,\* S.H. Vermund, J. Ma, and R.J. Deckelbaum. Mt. Sinai Medical Ctr., Albert Einstein College of Medicine/ Montefiore Medical Ctr., and Columbia University, New York, NY.

To assess the endemic prevalence of parasitic infections in day care centers (DCC), a cross-sectional study of intestinal parasites was carried out at an urban DCC not experiencing a diarrhea outbreak in September, 1987. The children were mostly white and upper middle class, and were cared for in age-defined cohorts. All stools were examined promptly using zinc sulfate stool concentration and trichrome staining, and sedimentation in distilled water and modified cold Kinyoun staining.

Results:

Age Group	#Tested	#Pos.(%)	Parasite (#)
Infants	10	3 (30)	<u>Cryptosporidium</u> (2); <u>Giardia</u> (1)
Toddlers	12	4 (33)	<u>Cryptosporidium</u> (4)
Preschool	9	1 (11)	<u>Giardia</u> (1)
Total	31	8 (26)	There were no mixed infections.

All 14 staff members were examined; 2 (14%) were positive, 1 each for Cryptosporidium and Blastocystis.

Asymptomatic cryptosporidiosis, found in 19% of the children, is a common occurrence in at least 1 urban DCC. While benign cryptosporidiosis has been described in India and Liberia, the present study is the first report of common, asymptomatic infection in DCC children from an industrialized nation.

ASTMH POSTER I

- 108 EFFECT OF RECOMBINANT TUMOR NECROSIS FACTOR AND INTERLEUKIN-1 TREATMENT ON THE INTRACELLULAR DEVELOPMENT OF EIMERIA TENELLA. M.H. Kogut and C. Lange. Rutgers University, New Brunswick, NJ 08903.

We have previously reported that a 16-24h treatment of Madin-Darby bovine kidney (MDBK) cells with recombinant human TNF- $\alpha$  significantly increased the susceptibility of the cells to invasion by sporozoites of the coccidial parasite, E.tenella, whereas the identical treatment with recombinant human IL-1 had no effect. The purpose of the present study was to determine whether continued treatment of these cells had any effect on the intracellular development of this parasitic protozoan. Treating the MDBK cells for 24h before infection and for 48 h after infection with 2-500U/ml of r-TNF- $\alpha$  had no effect on the development of E.tenella. However, this same treatment protocol using 10-25 U/ml of r-IL-1 significantly inhibited the development of E.tenella sporozoites by 36-51%. We found no synergistic effect with TNF and IL-1, TNF and r-bovine IFN- $\gamma$ , nor rIL-1 and r-IFN- $\gamma$ . Neither TNF- $\alpha$ , IL-1, nor IFN- $\gamma$  had a direct effect on extracellular sporozoites. These results taken together with our earlier studies provide evidence for a possible cytokine cascade involved in the protective mechanisms against coccidiosis.

- 109 THE NUDE MOUSE AS A MODEL FOR CRYPTOSPORIDIOSIS IN THE IMMUNODEFICIENT HOST.

Pentella M.A., Kwa B.H.,\* Vickery A.C. Lakeland Regional Medical Center, Lakeland, FL and the University of South Florida, Tampa, FL.

Cryptosporidium spp. is recognized as a significant pathogen in immunocompromised individuals, such as AIDS patients. The congenitally athymic nude mouse, which lack T-dependent immune responses, is therefore an ideal model for the study of cryptosporidiosis. In this study, an isolate of Cryptosporidium spp. was recovered from a 7 day-old calf with diarrhea and purified using a Percoll gradient. Fifteen female Balb/C nude, (nu/nu) mice and five heterozygous (nu/+) littermates were infected with  $10^7$  oocysts each, using a stomach tube. Fecal samples were collected twice weekly during the course of the infection. The fecal samples were concentrated and oocysts examined using phase contrast microscopy and fluorescent antibody staining. Mortality for the nude mice began three weeks after infection, the longest survival being twelve weeks. All heterozygotes recovered from the infection. The presence of specific anti-Cryptosporidium IgA antibody was 1:64 in nudes and 1:256 in heterozygotes. Postmortem histological examination of the infected mice revealed typical intestinal lesions.

## ASTMH POSTER I

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Diagnosis of Experimental Murine Toxoplasmosis Utilizing a *T. gondii* cDNA probe.  
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College of Medicine, Bronx, N.Y.

*Toxoplasma gondii* is a major cause of opportunistic CNS lesions in AIDS patients. There is an urgent need for a noninvasive, specific and rapid diagnostic test for this infection as definitive diagnosis at present requires brain biopsy. A cDNA probe for this organism has been made, which has demonstrated efficacy in a murine model of toxoplasmosis. *T. gondii* were purified from L<sub>6</sub>E<sub>9</sub> myocytes. They were disrupted in guanidium isothiocyanate and *Toxoplasma* mRNA isolated by affinity chromatography. A cDNA lambda gt11 library was prepared and screened for *Toxoplasma* specific clones using polyclonal antitoxoplasma antisera. Of the ~10<sup>6</sup> *Toxoplasma* specific cDNA clones generated, ten recombinant independent clones were selected at random and screened as possible diagnostic reagents. For these studies DNA was prepared from tissues of CD1 mice at varying intervals following infection with RH strain *T. gondii*. One of these recombinant cDNAs (clone #5, ca. 600 bp.) proved a particularly sensitive and specific diagnostic probe. Clone #5 readily and exclusively identified *Toxoplasma* DNA sequences in Southern blot analysis of Eco RI restricted total DNA extracted from the murine liver and spleen as early as three days post infection. Detection sensitivity was ~10 pg of clone #5 specific complimentary sequence, i.e., ~1 ng of total *Toxoplasma* DNA corresponding to ~ 10,000 organisms. Sequence analysis of clone #5 is in progress and will provide the data by which the level of sensitivity of this diagnostic test can be enhanced still further by taking advantage of the amplification potential of the polymerase chain reaction.

111 COMPARATIVE EVALUATION OF IFA AND EIA TECHNIQUES IN THE SEROLOGICAL  
DIAGNOSIS OF TOXOPLASMOSIS: DIFFICULTIES ASSOCIATED WITH SPECIMENS  
FROM IMMUNOCOMPROMISED PATIENTS. J.A. Kiehlbauch\*, R. Bacina, and  
T.R. Fritsche. University of Washington School of Medicine, Seattle, WA.

Two hundred sixty eight serum specimens from obstetrical, HIV seropositive, cardiac transplantation, and other patients were tested for the presence of IgG antibodies to *T. gondii* by both indirect fluorescent antibody (IFA; Microbiological Research Corporation) and enzyme immunoassay techniques (EIA; Abbott Laboratories and Sigma Diagnostics). Specificity for both EIA tests compared with IFA was 100%; sensitivity was 67% and 79% for the Abbott and Sigma tests, respectively.

Eighty-nine percent (25/28) and 86% (37/43) of the discrepant results (positive IFA; negative EIA) occurred in cardiac transplant patient specimens tested with the Sigma and the Abbott reagents, respectively. All specimens obtained from these patients and donors prior to transplantation were in agreement. In the post-transplant group significant titer rises (as much as 2-4 fold) were detected by IFA and correlated temporally with the administration of gamma globulin preparations. Increases were also evident with EIA techniques, but values did not cross the positive threshold. Subsequent investigation of 11 lots of commercially prepared gamma globulin found anti-*Toxoplasma* IgG levels from 1:128-1:512 by IFA. No IgM antibodies were detected in any lot. These results suggest that while the IFA is quite sensitive to passive immunization, EIA tests are affected to a much lesser degree. The clinician must be aware of the possibility of significant titer rises occurring in patients receiving immune globulin preparations, and interpret results appropriately in light of the test methodology used.

ASTMH POSTER I

112 MYCOBACTERIUM TUBERCULOSIS-SPECIFIC DNA PROBE: SEQUENCE ANALYSIS AND POLYMERASE CHAIN REACTION AMPLIFICATION FOR DIAGNOSIS.

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Plasmid probes, pMTb4 and pMTb5, were constructed by ligating a SmaI fragment of Mycobacterium tuberculosis DNA at the EcoRV and SmaI sites of pBR322 and pUC12, respectively. These recombinant plasmids hybridize to DNA from strains of M. tuberculosis, M. bovis, and M. bovis BCG but not with DNA from M. avium, M. intracellulare, M. scrofulaceum, Escherichia coli or human placenta. Southern transfers and restriction mapping indicate that pMTb4 contains the mycobacterial fragment in pMTb5 and an additional fragment of 484 basepairs. Sequence analysis of the one kilobasepair insert in pMTb4 shows that the guanosine plus cytosine ratio is rather high, ranging from 50% to almost 100% which is consistent with published reports of 70-75%. Using the sequence data, 21-basepair oligonucleotide primers were synthesized to amplify the common fragment using a polymerase chain reaction. In preliminary experiments with one nanogram of M. tuberculosis genomic DNA as template, a 400-basepair fragment could be resolved on an agarose gel stained with ethidium bromide. We will apply this technique to amplify DNA from small numbers of organisms present in tissue sections.

113 QUINIDINE AND QUININE AS MODELS OF THE NON-WEAK BASE ACTIVITY OF ANTI-MALARIALS. I.Y. Gluzman\*, P.H. Schlesinger, and D.J. Krogstad. Washington University, St. Louis, MO.

Our previous studies have shown that the ability of antimalarials such as chloroquine to raise vesicle pH in Plasmodium falciparum is greater than can be accounted for by their properties as diprotic weak bases. This "non-weak base" activity is presumably the reason why clinically useful antimalarials inhibit parasite growth at concentrations that do not affect mammalian cells. In these studies, we examined the antiplasmodial activities of quinidine and quinine (which are diastereoisomers) and measured their pKs in an effort to develop a structural correlate of non-weak base activity.

In vitro susceptibility testing (based on the inhibition of <sup>3</sup>H-hypoxanthine uptake) demonstrated that quinidine was approximately 3-fold more active than quinine against chloroquine-resistant parasites (ED<sub>50</sub>s of 128 vs 698 nM for the Indochina I strain). Quinidine was also more active than quinine against chloroquine-susceptible parasites (ED<sub>50</sub>s of 42 vs 102 nM for the Haiti 135 strain). The pKs of quinidine and quinine were measured by spectrofluorimetry, using emission at 330 nm and varying pH from 4 to 10 with 1 M acetic acid or NH<sub>4</sub>OH. As measured by this technique, there were no significant differences between the pKs of quinidine and quinine: the pK<sub>1</sub>s were 4.51 for quinidine and 4.61 for quinine; the pK<sub>2</sub>s were 8.57 for both quinidine and quinine. These results demonstrate that quinidine is more active than quinine against both chloroquine-susceptible and resistant parasites despite a lack of significant differences in their weak base properties (their pKs). These results suggest that the steric differences between quinidine and quinine may provide a key to structural prerequisites for non-weak base activity.

ASTMH POSTER I

114 REVERSAL OF CHLOROQUINE RESISTANCE IN PLASMODIUM FALCIPARUM FROM VARIOUS GEOGRAPHIC REGIONS.

A.M.J. Oduola,\* D.E. Kyle, S.K. Martin, and W.K. Milhous. Walter Reed Army Institute of Research, Washington, DC.

Reversal of chloroquine (CQ) resistance in Plasmodium falciparum by calcium antagonists in vitro was used to indicate if the mechanism of resistance is similar in various geographic regions. Intrinsic activities of CQ, desethylchloroquine (DESCQ) and combinations of each quinoline with calcium antagonists against field isolates and cloned strains of P. falciparum were evaluated by using a semiautomated microdilution technique. In vitro susceptibility profiles for each parasite were compared with the corresponding clinical treatment response and representative data are as follows:

	CQ IC <sub>50</sub> (ng/ml)		% Reduction
	Alone	+ modulator (1X10 <sup>-6</sup> M)	
Honduras	2.86	2.8	0.02
D-6 (Sierra Leone)	4.7	5.13	-0.09
Nigeria III/WRAIR	27.9	7.2	74.2
Cameroon I/WRAIR	40.2	7.85	80.4
Ghana I/WRAIR	41.4	12.7	69.3
W-2 (Indochina)	70.0	15.0	78.6
IEC-306 (Brazil)	87.4	20.9	76.0

The results indicate that the intrinsic activities of the CQ and DESCQ were potentiated by calcium antagonists only against resistant parasites and suggest that the predominant mechanism of resistance to CQ is the same in each geographic region where resistance exists.

115 CALCIUM CHANNEL BLOCKERS AS ADJUNCT TREATMENT OF MALARIA? W.K. Milhous, E.F. Boudreau, J. Freeman, L. Pang, D.E. Kyle, A.M.J. Oduola, C.J. Canfield, B.G. Schuster. Div of Exper Theraps, WRAIR, Washington, D.C.

Calcium channel blockers (CCB), such as verapamil (VER) reverse resistance to the antimalarial drugs chloroquine (Martin et al., 1987) and quinine (QUIN) (Kyle et al., 1988) in vitro. Since most of the clinically available CCB's have limited oral bioavailability and are toxic at required doses, the WRAIR is actively pursuing design of drug substances which will selectively reverse resistance in vivo without significant toxicity. Recognizing that other properties of CCB's, such as decreased red cell rigidity and increased cerebral perfusion, may exert a favorable influence on the hemodynamic consequences of severe and complicated malaria, we choose to quantitatively evaluate the triple drug combination VER, QUIN and tetracycline (TCN) in vitro. IC<sub>50</sub>'s were calculated from concentration response data generated for each drug alone and in combination at various fixed ratios of IC<sub>50</sub>. Fractional Inhibitory Conc (FIC) indices (1.0 = additivity, <0.5 = synergism) were calculated for each drug combination and are summarized in the adjacent table. With significant reductions in the toxicity of candidate CCB's, these findings suggest that, in addition to the potential for reversing the pathological hemodynamics of cerebral malaria, CCB's might also enhance the therapeutic index of the current treatment of choice, QUIN & TCN.

	FIFTY PERCENT INHIBITORY CONC.			COMBINATION FIC INDICES
	(in ng/ml)			
	VER	TCN	QUIN	
Drugs Alone	2744	17054	59.80	----
<u>Drugs Combined in Fixed Ratios of IC<sub>50</sub></u>				
1:1:0	1107	01107	-----	0.46
1:0:1	0899	-----	03.59	0.38
0:1:1	----	07686	30.74	0.96
1:2:3	0473	00945	05.67	0.31
3:2:1	0904	00603	02.40	0.39
1:3:2	0418	01255	03.34	0.27
3:1:2	0644	00215	01.71	0.28
2:1:3	0582	00291	03.49	0.28
2:3:1	0485	00727	00.96	0.22

- 116 **IN VITRO ACTIVITY OF DOXYCYCLINE IN COMBINATION WITH QUININE AGAINST BLOOD STAGES OF *Plasmodium falciparum***  
 R. G. TAYLOR,\* W. K. MILHOUS, D. L. BROWN, AND D. M. HOCHSTEDLER.  
 Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD, and Walter Reed Army Institute of Research, Washington, DC.

The current treatment of choice for multiple drug resistant (MDR) falciparum malaria (PF) is tetracycline (TCN) with quinine (QUIN). Doxycycline (DCN) has better oral bioavailability and longer serum half-life than TCN and has been used successfully for chemoprophylaxis of MDR malaria on the Thai-Burmese border (Pang 1987 Lancet I:1161). We have shown previously that DCN is approximately five times as effective as TCN *in vitro*. Therefore, we studied the combination of TCN or DCN with QUIN against two clones of PF to look for any significant interaction between DCN and QUIN *in vitro*. Fifty percent inhibitory concentrations (IC<sub>50</sub>) were determined by using <sup>3</sup>H-hypoxanthine incorporation as an index of parasite growth. Drug combinations were studied by mixing the two drugs in fixed ratios of predetermined IC<sub>50</sub>'s. Fractional inhibitory concentrations were calculated from the concentration response for TCN or DCN alone and in combination with QUIN. There was no significant interaction between doxycycline and quinine with regard to intrinsic antimalarial activity. Tetracyclines should be used cautiously for chemoprophylaxis of MDR malaria because the parasite may become resistant and side effects are possible (Bruce-Chwatt 1987 Lancet I:1487).

- 117 **DIETHYLDITHIOCARBAMATE (DDC) ACTS SYNERGISTICALLY WITH COPPER AS AN ANTIMALARIAL.**  
 D. Gray Heppner,\* Steven R. Meshnick, Allen Ranz, Shu-fang Lu, Ming-wei Qian and John W. Eaton. University of Minnesota, City College of New York, and Henan Medical University.

Prompted by the urgent need for new antimalarials, we have reexamined the antimalarial action of DDC, a metal chelator having special affinity for copper. Other metal chelators evidently exert antimalarial effect either by sequestering a metal essential for parasite development (e.g., deferoxamine B which is ineffective as the iron chelate - ferrioxamine) or by forming a possibly reactive chelate (e.g., 8-hydroxyquinoline which forms a toxic iron complex). Using normal human red cells and *P. falciparum* (FCR3) infected erythrocytes (obtained by the *in vitro* culture technique of Jensen and Trager), we find that DDC alone exhibits an ED<sub>50</sub> of approximately 10 μM when added to cultured *P. falciparum*. Whereas copper alone (up to 12 μM) has no effect, the presence of 5 μM copper will reduce the ED<sub>50</sub> of DDC to 3 μM, an effect in keeping with earlier observations showing enhanced fungicidal activity of DDC following complexation with copper. This DDC/Cu synergy may be exerted through the marked solubility of DDC/Cu in organic solvents; the partition coefficient of DDC/Cu complex in chloroform:water is >1000:1, suggesting that the chelate might be preferentially damaging to cellular membranes. Indeed, we find that DDC/Cu is selectively concentrated by normal human red cell membranes. Consonant with this, whereas neither DDC (up to 10 mM) or Cu (up to 100 μM) is hemolytic during brief incubation, the 2:1 chelate is rapidly lytic at concentrations as low as 50 μM. Our results suggest that DDC exerts maximal antimalarial effect in combination with copper. The mechanism underlying this apparent synergy is not known but may involve the membrane destabilizing effects of the chelate.

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118 A SYNERGISTIC DELETERIOUS EFFECT OF ASCORBATE AND COPPER ON THE DEVELOPMENT OF PLASMODIUM FALCIPARUM IN NORMAL AND IN G6PD DEFICIENT ERYTHROCYTES.

J. Colenser\*, E. Mrva\*, A. Cohen\*, P. Saltman\*\*, M. Chevion\*\*\*  
Depts. \*Parasitology and \*\*\*Cellular Biochemistry, the Hebrew University, Jerusalem, Israel and Dept. \*\*Biology, University of California, San Diego, USA.

Malaria parasites grown in G6PD(-) erythrocytes rather than in normal (G6PD(+)) erythrocytes are more sensitive towards oxygen derived free radicals. Copper and iron are effective enhancers of the oxidant stress induced damage.

In this work we examined the development of P. falciparum in G6PD(+) and G6PD(-) erythrocytes treated with ascorbate and copper. Experiments were performed in two modes: a) non-infected erythrocytes were pretreated and only subsequently infected with P. falciparum. b) parasitized erythrocytes were treated. The development of the parasites was followed by determining parasitaemias and by measuring the uptake of radioactive hypoxanthine (Hx) in the cultures.

There were minor suppressive effects of the pretreatment with ascorbic acid, copper or their combination on the development of the parasite in G6PD(+) erythrocytes. In G6PD(-) cells the effects were dose-dependent and much more pronounced. A synergistic effect could be seen in G6PD(-) cells: i.e. while copper or ascorbate alone caused 2% or 5% inhibition of Hx incorporation, respectively, their combination resulted in 45% inhibition.

Dose dependent suppressive effect of ascorbate or copper was demonstrated following treatment of parasitized erythrocytes (G6PD(+) or G6PD(-)). In those cells a synergistic effect between ascorbate and copper was expressed in the reduction in Hx incorporation and parasitaemias as well as by the retardation of maturation of the plasmodia.

The involvement of transition metals in the deleterious effects was further examined by the addition of the chelator Detapac to the parasitized erythrocytes. Detapac (100µm) markedly reversed the inhibitory effect of ascorbate, copper and their combination. The results suggest that reactive-oxygen species induced by transition metals are involved in the inhibition of the development of P. falciparum and provide an additional indication for the advantage of G6PD(-) individuals in relation to malaria.

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EVALUATION OF EXPERIMENTAL COMPOUNDS FOR CAUSAL PROPHYLACTIC ACTIVITY AGAINST MALARIA IN SPOROZOITE INOCULATED MICE. R.G. May, Ager, A.L. Department of Microbiology and Immunology, University of Miami.

Over 6,000 experimental compounds of a wide variety of chemical types were tested for causal prophylactic activity in mice inoculated with Plasmodium yoelii sporozoites. Residual activity was assessed by administering each compound both orally (80mg/kg) and subcutaneously (80mg/kg) to 3 groups of mice either 21 days, 14 days or 7 days prior to inoculation with  $2.5 \times 10^5$  P. yoelii sporozoites. Blood films of each mouse were stained with Giemsa and observed for the presence of parasites. 371 compounds displayed varying degrees of residual activity for 21 days and were tested for longer periods in increments of 7 days until residual activity ceased. 35 days after drug administration 18 2,4-diaminoquinazolines remained active. By day 56 only 4 had activity and by day 70 one was active. Two 7-aminoquinolines were active 49 days and one of these was active past 63 days. One diaminodiphenyl-sulfone was active for 42 days and one was active past 49 days. Two 2,4-diaminopteridines were active past 56 days. Three acridinoneines were active for 35 days and one was active for 42 days. Three acridines were active for 42 days.

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120 EFFECT OF MEFLOROQUINE AND QINGHAOSU ON THE SPOROGENIC CYCLE OF PLASMODIUM BERGHEI ANKA IN ANOPHELES STEPHENSI MOSQUITOES. R.E. Coleman <sup>\*2</sup>, J.E. Vaughan <sup>3</sup>, D. Hayes <sup>1</sup>, M. Hollingdale <sup>1</sup>, M. Plein <sup>1</sup> and V.E. Do Rosario <sup>1</sup>.

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- (2) Dept. Entomology WRAIR, Washington DC
- (3) School of Medicine, Univ. Maryland Baltimore, MD

Anopheline mosquitoes may ingest anti-malarial drugs during feeding. This study was designed to assess the impact of mefloquine (MFQ) and qinghaosu (QHS) on 1) oocyst development, 2) presence of sporozoites in the haemolymph and 3) subsequent sporozoite invasion of the salivary glands. Outbred Dominion mice were inoculated with MFQ (5 or 25mg/kg b.w.) or QHS (10 or 50 mg/kg b.w.) 4 days after they had been inoculated with Plasmodium berghei ANKA. Control mice received only PBS. Anopheles stephensi mosquitoes engorged on the mice 2 hours after administration of the drug. From day 8 onwards, 15 mosquitoes from each group were dissected every other day, until the conclusion of the experiment.

Low dosages of MFQ and QHS resulted in an earlier appearance of sporozoites in the haemolymph, and subsequently, in the salivary glands than in control fed mosquitoes. High dosages of MFQ resulted in a delay in sporozoite invasion of salivary glands, whereas high dosages of QHS had no effect.

This work was supported by a WHO grant # 870105 (VEdR), by a National Research Council Fellowship (REC) and a NIH/AI 17828 (JAV).

121 TRANSIENT CONTINUATION OF CHLOROQUINE-SENSITIVE PLASMODIUM FALCIPARUM PARASITEMIA IN VOLUNTEERS RECEIVING CHLOROQUINE THERAPY. D.A. Herrington,\* D.F. Clyde, J.R. Murphy, S. Baqar and J.R. Davis. Center for Vaccine Development, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD.

Standard oral chloroquine (CQ) therapy was administered for 3 days commencing at the onset of patency of known CQ-sensitive P. falciparum infections which had been induced by an extremely heavy sporozoite inoculum. Such therapy was curative; however, for most of the 8 volunteers, peak parasitemias occurred 2 to 5 days after initiation of CQ therapy. In some individuals, peak parasitemia was nearly 100 fold greater than that at initiation of therapy. This pattern of infection was surprising in view of the known capacity of CQ to rapidly eliminate CQ sensitive parasites developing within red blood cells. Examination of the time course and magnitude of parasitemias showed that some of the late peaks could not be explained by continued growth of asexual erythrocytic parasites since the pattern of infection did not conform to known characteristics of the erythrocytic cycle. Possible explanations include the introduction of additional CQ-sensitive parasites into the peripheral circulation after the initiation of therapy from sequestered foci within capillary beds or via the delayed rupture of some exoerythrocytic schizonts.

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- 122 EFFICACY OF MALARIA PROPHYLAXIS IN PREGNANT WOMEN: THE ROLE OF PATIENT COMPLIANCE VERSUS PARASITE SENSITIVITY. J.W. Wirima,\* D.L. Heymann, and R.W. Steketee. Malawi Ministry of Health, Lilongwe, Malawi; International Health Program Office and Malaria Branch, CDC, Atlanta, GA.

Because of high rates of *P. falciparum* parasitemia among women enrolled in an antenatal clinic malaria prophylaxis program, we investigated the protective efficacy of chloroquine (CQ) prophylaxis by assessing patient compliance and *P. falciparum* sensitivity to CQ. 642 women enrolled in the voluntary CQ prophylaxis program (unsupervised ingestion of 300 mg/wk) were examined for peripheral parasitemia and the presence of CQ-metabolites in their urine. An additional 119 pregnant women were enrolled in a supervised prophylaxis program (observed drug ingestion) and were examined after at least 4 weekly doses of CQ (300 mg/wk). Attack rates (AR) of parasitemia in women in the supervised program were 34% compared to a 44% AR in non-compliant women (those without CQ in their urine) in the unsupervised program, suggesting a protective efficacy of CQ prophylaxis of 23%. Among women on unsupervised prophylaxis, 22% took the drug, as evidenced by the presence of CQ in urine; because of the low compliance rate the overall protective efficacy of CQ prophylaxis was 5%. CQ prophylaxis in this area of CQ-resistant malaria has low efficacy due to a combination of parasite resistance and poor patient compliance. Supported by CCCD USAID PASA BAF 0421 PHC 22333.

- 123 CHLOROQUINE AND MEFLOQUINE USED FOR MALARIA PROPHYLAXIS DURING PREGNANCY. R.W. Steketee,\* J. Wirima, D.L. Heymann, C. Khoromana, and J.G. Breman. Malaria Branch and International Health Program Office, CDC, Atlanta, GA; Ministry of Health, Malawi.

Antimalarial chemoprophylaxis following an initial treatment dose is recommended to prevent the adverse effects of *P. falciparum* malaria in pregnant women. Chloroquine (CQ) has been most widely used but the spread of CQ-resistance may limit the efficacy of this drug. In Malawi, we compared the efficacy of various antimalarial drug regimens in pregnant women to prevent parasitemia and associated low birthweight. From September 1987 to May 1988, 1384 pregnant women were enrolled in 1 of 4 chemoprophylactic regimens: (a) CQ 25 mg/kg followed by weekly CQ 300 mg, (b) monthly CQ 25 mg/kg, (c) weekly CQ 300 mg and CQ 25 mg/kg for fever illness, and (d) mefloquine (MQ) 750 mg followed by weekly MQ 250 mg. Among women in their first pregnancy the drug regimens were, respectively, 71%, 69%, 60%, and 96% effective in maintaining women aparasitemic. A total of 395 study women on regimen a or b and 1,308 comparison women (those attending antenatal clinics but not receiving supervised antimalarial prophylaxis) delivered singleton live born children and were evaluated for placental malarial infection and birth weights. Placental infection rates were similar in study and comparison women (respectively, 34% and 36% in primiparas, 22% and 24% overall); birthweights were not significantly different between the study and comparison newborns. CQ prophylaxis showed limited efficacy in this area of CQ-resistance and MQ is still under evaluation. Supported by CCCD USAID PASA BAF 0421 PHC 22333.

ASTMH POSTER I

- 124 MALARIA CHEMOPROPHYLAXIS STUDY AMONG PEOPLE LIVING IN A MALARIA ENDEMIC AREA OF THE PHILIPPINES. R. Oberst\*<sup>1</sup>, L. Laughlin<sup>1</sup>, N. Sy<sup>1</sup>, A. Alcantara<sup>1</sup>, L. Padre<sup>1</sup>, C. Manaloto<sup>1</sup>, M. Santos<sup>2</sup>, and C. Echeverri<sup>2</sup>. U.S. Naval Medical Research Unit No. 2<sup>1</sup> and Malaria Control Service, Department of Health, Republic of the Philippines<sup>2</sup>, Manila, Philippines.

An endemic area for malaria on the island of Palawan was chosen for conducting chemoprophylaxis trials using either chloroquine, Fansidar<sup>®</sup>, mefloquine or a placebo. To our knowledge no information is available on malaria prophylaxis with these drugs in the Philippines although both in-vitro and in-vivo resistance to chloroquine have been documented. Fansidar<sup>®</sup> resistance has also been shown in-vivo and in-vitro resistance to mefloquine has been suggested.

Patients who had two negative blood smears one week apart whether initially negative or post treatment, were enrolled and assigned randomly to one of four drug groups (including a placebo) within three age groups; 5-8, 9-15, and  $\geq$  16 years. There were initially 651 patients enrolled, 81 of whom dropped out during the course of the study. While chloroquine patients were unprotected against Plasmodium falciparum (20/111 patients), Fansidar<sup>®</sup> patients were unprotected against P. vivax (13/112 patients). There were no cases of P. vivax or P. falciparum in the 112 patients on mefloquine, no cases of P. falciparum among those on Fansidar<sup>®</sup>, and no cases of P. vivax among those on chloroquine. Among the 165 patients on placebo there were 50 cases of P. falciparum and 30 cases of P. vivax. Results are being interpreted by survival analysis statistics over the 3 month period of the study.

- 125 WHOLE BLOOD LEVELS FOR MEFLOROQUINE AND ACID METABOLITE IN PREGNANT WOMEN IN MALAWI. L. Patchen,\* L. Slutsker, S. Williams, J. Wirima, and R. Steketee. Control Technology Branch and Malaria Branch, CDC, Atlanta GA; and Ministry of Health, Malawi.

Mefloquine (MQ) is being evaluated as an alternative drug to prevent the adverse effects of chloroquine-resistant Plasmodium falciparum during pregnancy. To determine the appropriate MQ dosages, MQ and the acid metabolite (MMQ) levels were determined in aparasitemic pregnant women given a prophylactic regimen of 250 mg weekly (M250) and in parasitemic pregnant women given a single 750 mg therapeutic dose (M750). Assays for MQ and MMQ were performed on whole blood samples by high performance liquid chromatography, with detection limits in 0.2 ml samples of 50 ng/ml and 65 ng/ml, respectively. Specimens were collected for the M750 group (n=10) at 4 hours and days 2 (D2), 7, 14, and 21 after treatment. Whole blood levels for MQ at 4 hours ranged from 926-2096 ng/ml, and decreased to 369-851 ng/ml by D21. Levels for MMQ at D7, ranged from 252-949 ng/ml, and decreased to 182-414 ng/ml by D21. Estimated mean elimination half-lives for MQ and MMQ after peak levels were 13 and 15 days, respectively. Samples for the M250 group (n=11) were collected weekly just prior to the next scheduled MQ dose. On D28, whole blood levels ranged from 669-931 ng/ml for MQ and 207-456 ng/ml for MMQ. After initial clearance of parasites, all treated women remained aparasitemic through D28. These data are being used to determine if pregnancy alters MQ pharmacokinetics and to evaluate therapeutic and prophylaxis dosages necessary during pregnancy for P. falciparum protection in chloroquine-resistant regions. Supported in part by CCCD USAID PASA BAF 0421 PHC 22333.

- 126 LACK OF EFFICACY OF PYRIMETHAMINE PROPHYLAXIS IN PREGNANT NIGERIAN WOMEN. B.L. Nahlen,\* A. Akintunde, T. Alakija, P. Nguyen-Dinh, O. Ogunbode, L.D. Edungbola, O. Adetoro, and J.G. Breman. Malaria Branch, CDC, Atlanta, GA; University of Ilorin, Ilorin, Nigeria.

In Nigeria, weekly pyrimethamine (PYR) has been the drug of choice to prevent adverse effects of *Plasmodium falciparum* during pregnancy. To evaluate the efficacy of PYR in pregnant women, *in vivo* and *in vitro* drug studies were conducted in Ilorin, Nigeria, during January-May, 1988. Prevalence of parasitemia in women attending antenatal clinics was 41% in primigravidae and 18% in multigravidae ( $p < 10^{-6}$ ). Of 88 pregnant women given PYR 25 mg, 37 (79%) primigravidae and 22 (54%) multigravidae remained parasitemic on day 7 ( $p < 0.03$ ). All PYR failures were given chloroquine (CQ) 300 mg and were aparasitemic on day 7. Six of 10 *in vitro* tests demonstrated resistance to PYR and cycloguanil; all 23 *in vitro* microtests indicated sensitivity to CQ. To determine the *in vivo* causal prophylactic efficacy of PYR, 70 pregnant women were cured with CQ 25 mg/kg; half received PYR 25 mg weekly beginning on day 7 and had weekly blood smears examined, while the other half served as non-treated controls. Four (11%) women taking PYR and 5 (14%) controls had detectable parasites during the 12-week follow-up period. Primigravidae were more likely to be parasitemic and less likely to clear parasitemia on PYR prophylaxis. Thus, PYR has limited efficacy as suppressive or causal prophylaxis in pregnant women in Ilorin. Despite widespread belief in PYR efficacy, an alternative drug such as CQ should be evaluated for prophylaxis of pregnant women in this area. Supported by USAID PASA BAF 0421 PHC 22333.

- 127 KINETICS OF INTRAMUSCULAR AMOPYROQUIN\* IN HEALTHY SUBJECTS AND MALARIAL PATIENTS

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The disposition of amopyroquin (ApQ) was investigated in 10 healthy volunteers after a single 2 mg/kg intramuscular dose of ApQ base. After an absorption phase of 15 min, plasma levels of unchanged drug decreased following a tri-exponential model with a terminal  $t_{1/2}$  of  $130 \pm 90$  h.  $V_d/F$  and  $Cl/F$  were  $240 \pm 80$  l/kg and  $2060 \pm 1160$  ml/min respectively. The renal clearance (urine of the first 48 h) was  $120 \pm 100$  ml/min and represented 6% of the systemic clearance. Twenty-two *P. falciparum* malarial patients (selected from a larger therapeutic study) were followed for parasitemia clearance at day 7, *in vitro* drug sensitivity of the isolates and ApQ blood levels after treatment with the following regimens: (1) 3 mg/kg; (2) 6 mg/kg and (3) 6 + 3 mg/kg at 24 h interval. In all patients, IC50 of isolates ranged from 10 to 780 nmol/l for chloroquine and from 4 to 25 nmol/l for amopyroquin. Parasitemia was cleared in only 1 out of 6 patients and ApQ levels at 48 h ranged from 62 to 123 nmol/l with the regimen (1), in 4 out of 7 patients and ApQ levels at 24 h ranged from 131 to 237 nmol/l with regimen (2) and in 7 out of 9 patients and ApQ levels at 48 h ranged from 106 to 236 nmol/l with regimen (3). The modelled concentration-time profiles indicated that a regimen of 12 mg/kg (in 2 or 3 injections) could maintain higher blood levels (at least 200 nmol/l) for a longer time (about 100 h) than previous regimens (from 15 to 72 h).

\* Warner-Lambert, Parke Davis, France.

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128 A BIOASSAY FOR DETERMINING CYCLOGUANIL CONCENTRATIONS IN HUMAN PLASMA USING PLASMODIUM FALCIPARUM AS THE TEST ORGANISM.  
 C. Lambros\*, V. Navaratnam, and G.E. Leiwis Jr. U.S. Army Medical Research Unit-Malaysia, Institute for Medical Research, Kuala Lumpur and National Drug Research Center, Penang, Malaysia.

A renewed clinical interest in the biguanidines, proguanil and chlorproguanil, has developed as a result of the prevalence of drug-resistant *P. falciparum*. These compounds exert their antiplasmodial activity after metabolic conversion to a triazine. Although analytical methods are available to determine plasma concentrations of proguanil, chlorproguanil, and the active metabolite of chlorproguanil (chlorcycloguanil), the active metabolite of proguanil (cycloguanil) has proved difficult to measure. A bioassay was developed to measure plasma concentrations of cycloguanil using *P. falciparum* as the test organism. The bioassay consists of: a cycloguanil-sensitive isolate of *P. falciparum* maintained in human plasma; a culture medium with reduced amounts of p-aminobenzoic acid and folic acid; and the use of the radioisotope microdilution method to determine the 50% inhibitory concentration (IC-50), substituting plasma concentration for drug concentration. Total incubation time was 64 hrs. Using pre-dose plasma samples containing known amounts of cycloguanil ranging from 20-150 ng/ml, the reproducibility of the assay was found to be very good. Our results document that a practical bioassay to measure plasma concentrations of cycloguanil is now a reality.

129 CHLOROQUINE AND FANSIDAR® RESISTANT FALCIPARUM MALARIA ACQUIRED IN LIBERIA BY AN AMERICAN TRAVELER.  
 P.F. Pierce, A.M.J. Oduola, D.E. Kyle, L. Gerena, L.C. Patchen, and W.K. Milhous\*. Georgetown Univ Med Ctr & WRAIR, Washington, DC & CDC, Atlanta, GA.

Implementation of an intensified surveillance program to detect drug resistant *falciparum* malaria has demonstrated that non-immune American travelers can serve as sentinel cases in predicting the increasing prevalence and severity of chloroquine (CQ) resistance in west Africa. A 41 yr/old male, who was born in Liberia but lived in the U.S. for the past 25 years, began faithful CQ prophylaxis 2 weeks prior to departure for Liberia and continued until his day of admission at Georgetown Medical Center. He had traveled to Liberia by airplane with a 3 hr stop in Dakar and spent 2 weeks in ZorZor and 1 week in Monrovia. He developed fever, chills and headache on his day of return to the U.S. Upon admission he was diagnosed as having *falciparum* malaria and 30 hrs after treatment with Fansidar® [sulfadoxine (SDX) & pyrimethamine (PYR)] he became febrile with positive smears. He was subsequently treated with quinine and tetracycline and has since remained afebrile. Whole blood levels of CQ (368 ppb) and its metabolite, desethylchloroquine (113 ppb) confirmed CQ prophylaxis and 27 ppm of SDX was detected in whole blood 30 hours after Fansidar® treatment. Parasites were successfully cultured *in vitro* and fifty percent inhibitory concentrations (ng/ml) calculated using the method of Milhous et al., 1985, with simultaneous control clones (Oduola et al., 1988):

	CQ	DECQ	PYR	SDX
Liberia Patient Isolate	28.14	500	14.88	22,092
Susceptible African Control Clone	03.76	005.20	00.03	32.51
Resistant Indochina Control Clone	37.40	236.37	39.49	49,083

This case report and others currently under evaluation confirm the presence of drug resistant *falciparum* malaria in Liberia and underscore the importance of applying the appropriate parasitologic and therapeutic criteria to document drug resistance.

ASTMH POSTER I

- 130 MALARIA DRUG THERAPY IN ESMERALDAS PROVINCE, ECUADOR. B.L. Nahlen, J.D. Sexton,\* T.K. Ruebush II, I. Caicedo, G. Macias, E.B. Hayes, and J.M. Stewart. Divisions of Parasitic and Viral Diseases, CDC, Atlanta, GA; Servicio Nacional de Erradicacion de la Malaria (SNEM), Ecuador.

Development of antimalarial drug use policies requires a knowledge of antimalarial drug efficacy and patient treatment-seeking behavior. In Ecuador, chloroquine-resistant Plasmodium falciparum (CRPF) is thought to be a problem, especially in Esmeraldas Province, which has only 6% of the population who live in malarious areas but reports 70% of P. falciparum cases. In 1987 we conducted in vivo drug sensitivity studies in the cities of Esmeraldas and San Lorenzo. At the same time, surveys to determine residents' knowledge about antimalarial therapy and their behavior in seeking treatment for malaria were conducted in 5 communities near San Lorenzo. In vivo results showed that of 52 P. falciparum infections, 24 (45%) were chloroquine (CQ) resistant, but CQ was still effective in reducing fever. Of 125 persons interviewed during the surveys, 96% had used CQ provided by SNEM, but only 6% knew the correct CQ dose and 70% reported side effects. Fansidar<sup>R</sup> had been used by 54% of informants, and 28% reported side effects. Eighty-two percent believed Fansidar<sup>R</sup> was more effective than CQ. Of 73 informants reporting a recent malarial illness, 24 (33%) received CQ at a SNEM laboratory; most of the remaining patients purchased antimalarials in local stores. SNEM should continue to use CQ as primary therapy and reserve Fansidar<sup>R</sup> for CRPF cases, educate the population about antimalarial therapy and CRPF, and establish a drug sensitivity monitoring system. Supported by USAID, PASA LAC-0049-P-HC-6036-00.

- 131 EFFICACY OF A 3-DAY ORAL QUININE TREATMENT FOR P. FALCIPARUM MALARIA IN MADAGASCAR. C. Chouquet\*, J.P. Lepers, P. Deloron, F. Verdier, J.A. Ramanamirija, G. Jaureguiberry, P. Coulanges. INSERM U13/INRA, Paris, France; Institut Pasteur de Madagascar, Antananarivo; Hop. StLouis, Paris, France

The recent extension of parasitologic resistance to chloroquine (C) of P.falciparum in Africa might be followed in the near future by a decline of its clinical activity. Oral quinine (Q) would be a valuable alternative therapy if efficacy could be achieved with a shorter treatment than the usual scheme (7 to 10 days). An in vivo and in vitro study of the activity of a 3-day oral Q (8 mg/kg/8 hours) therapy of P. falciparum clinical malaria was conducted in Madagascar in 1988. 39 individuals > 10 years old with uncomplicated P.falciparum malaria were enrolled. Clinical and parasitologic examination were performed before each drug intake, daily until D7 and weekly until W4. To assess the respective role of recrudescences and reinfections in the reappearance of parasites in blood between D7 and W4, we simultaneously followed a control group of 48 individuals presenting on D0 with a negative blood smear. At enrolment, the GMPD in the treated group was 8739 parasites/ $\mu$ l, the mean axillary temperature was 38.29°C and 68% of the subjects were febrile (temperature > 37.5°C). C intake prior to enrolment, determined in plasma by HPLC, was demonstrated in 63% of the individuals, but to low levels (mean of C and metabolites: 76.1  $\mu$ g/l). Q was administered by one of us in 68% of the treatment events, and plasma levels of Q and metabolites were assessed on D2 (8 hours after the 6th dose) by double extraction fluorescence. Mean concentration was 7.45 mg/l ( $\pm$  3.57) and 5 individuals had insufficient Q levels (< 3 mg/l). Successful in vitro semi-microtests were performed with 19 isolates. 63% were resistant to C (CI50 > 100 nM), all being sensitive to mefloquine and Q. Parasites were detected in blood of 6% of the individuals on D3, and in none on D4. All subjects were still parasitemic on D7. The mean parasite clearance time was 58.9 hours. Mean temperature fell under 37°C on D2, with 14% of the individuals being still febrile. P. falciparum parasites reappeared in the blood of 9%, 45%, and 55% of the individuals by W2, W3, and W4, respectively. In the control group, the cumulative percentages of parasitemic subjects were 11%, 21%, 29%, and 39% on W1, W2, W3, and W4, respectively. Blood parasites were significantly more frequently observed by W3 and W4 in the treated group than in the control group, but this difference disappeared when the 5 subjects with insufficient Q blood level on D2 were excluded. These results demonstrate that oral quinine given over 3 days could be an alternative therapy of choice of P. falciparum malaria in areas of resistance to chloroquine.

SYMPOSIUM: THE WATER AND SANITATION DECADE (1981-1990):  
ITS IMPACT ON TROPICAL DISEASE

- 132 THE DECADE IN PERSPECTIVE. A. Rotival. UNDP/WHO Coordinator, International Drinking Water Supply and Sanitation Decade, Geneva, SWITZERLAND.
- 133 IMPACT ON DIARRHEAL DISEASES: NEW RESULTS AND IMPROVED METHODOLOGY. R. Feacham. Population and Human Resources Department, The World Bank, Washington, DC.
- 134 MOVIE: "GUINEA WORM: THE FIERY SERPENT"
- 135 SCHISTOSOMIASIS CONTROL AND THE DECADE: ECOLOGICAL APPROACHES TO EVALUATION. F. DeWolfe Miller. School of Public Health, University of Hawaii, Honolulu, HI.
- SUMMARY. D.R. Hopkins.

ABSTRACT

The United Nations International Drinking Water Supply and Sanitation Decade (1981-1990) has focused international attention on the provision of safe drinking water and sanitation facilities to 1.5 billion persons (1980 baseline) without access to clean drinking water, and 1.8-1.9 billion with no sanitation services. By mobilizing resources and political commitment, the Decade program has been successful in increasing the availability of water and sanitation to millions in the Third World. In this process, the Decade has had major impact on control of diseases transmitted by, or in relation to, water. With little more than two years remaining until the end of the Decade, it is appropriate to review some of the specific accomplishments, as well as the problems remaining. The symposium will present an overview of the achievements of the Decade and critically examine data evaluating the impact of the program on diarrheal diseases, dracunculiasis and schistosomiasis.

D: ARBOVIROLOGY - EPIDEMIOLOGY AND PATHOGENESIS

136 SEROPREVALENCE OF ANTIBODY (AB) TO ST. LOUIS ENCEPHALITIS (SLE) VIRUS IN AN ADULT MEDICAL AND PEDIATRIC OUTPATIENT POPULATION IN LOS ANGELES COUNTY (LAC).

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The first urban case of SLE ever reported in the western US occurred in LAC in 1983 and was followed in 1984 by an outbreak in Southern California of 26 confirmed cases. To assess the endemicity of SLE virus in LAC, 4,936 serum samples were collected anonymously from outpatients at 23 privately operated clinics in LAC between October and December 1986. Demographic data collected and linked anonymously to each specimen included age, sex, race, census tract (CT), zip code, health district, and years of residence at current address and within LAC. A population adjusted, geographic and age stratified subsample of 1,803 specimens was screened for IgG Ab to SLE virus by an enzyme-linked immunosorbent assay. Seroreactive specimens were confirmed with a neutralization antibody (NA) test. Cross-NA testing to dengue and yellow fever is pending. Twenty-eight sera (1.6%) were positive by NA test. Seropositives were significantly older (mean=46.9 yrs, SD=18.0;  $p<.001$ ) than seronegatives (mean=35.4 yrs, SD=18.6). The rate of SLE Ab among Asians was 3.9% (4/102), Hispanics 2.3% (12/511), whites 1.3% (9/700), and blacks 0.28% (1/353). Twenty-one seropositives (75%) had resided for 5 years or more at their current address. Seropositives were more likely to have resided in the same CT or a CT adjacent to a clinical and serologically diagnosed case of SLE during the period 1983-1987 (Odds Ratio=2.41,  $p<.02$ , CI 1.12,5.08). No difference in sex or length of residence in LAC was observed. We conclude that a highly susceptible population resides in LAC; the current distribution of infection appears to be focal; and, the low overall seroprevalence suggests that the virus may have only recently been introduced into LAC.

137 EPIDEMIOLOGIC ASPECTS OF A WESTERN EQUINE ENCEPHALITIS EPIDEMIC IN THE UNITED STATES, 1987.

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Western equine encephalitis (WEE) in 1987 produced 40 human and 173 equine cases in the largest outbreak reported from the United States since 1977. Cases were reported principally from western plains and mountain states. Cases were recognized initially in southern Texas and in subsequent months the epizootic appeared to spread in a northward advance. Seventeen St. Louis encephalitis (SLE) cases also were reported in 1987; all were from western states with evidence of WEE virus transmission. Active surveillance for human disease in Colorado led to the detection of 30 cases, one fatal. WEE incidence rates in the state were higher in males and increased directly with age. Risk was lowest in highly urbanized areas of the state. Case-control methods were used for the first time to assess risk factors for acquiring WEE. Protection in 21 cases and 33 age-sex matched controls was associated with air-conditioned residences and preference for watching television.

D: ARBOVIROLOGY - EPIDEMIOLOGY AND PATHOGENESIS

- 138 HANTAVIRUS INFECTIONS IN HUMANS AND COMMENSAL RODENTS IN SINGAPORE  
T.W. Wong,\* Y.C. Chan, Y.G. Joo, H.W. Lee, P.W. Lee, R. Yanagihara,  
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Singapore, Korea University, Seoul, National Institutes of Health,  
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To determine the extent of hantavirus infection in Singapore, serological studies using the indirect immunofluorescent antibody (IFA) test were conducted on commensal rodents and on human patients of four diagnostic groups. Virus isolation using a Vero E6 cell line was performed on hantaviral antigen-positive rodent lung tissue. Of 142 rodents and 3 insectivores studied, 37 (26%) were seropositive for IFA. Rattus norvegicus was the predominant species captured, with the highest species-specific seropositive rate of 32% (36 of 113). A hantavirus strain R36 was isolated from one *R. norvegicus*. Seropositive rates for human patients were: 8% respectively for dengue haemorrhagic fever suspects and for non-A non-B hepatitis patients, 3% for leptospirosis suspects and 2% for acute nephritis patients. Two patients had marked liver dysfunction but mild renal involvement. This hepatitis-like manifestation appear to be a clinical variant of hantavirus infection.

- MARBURG VIRUS: THE SEARCH AT KITUM CAVE.  
139 E.D. Johnson,<sup>1</sup> J. Morrill,<sup>1</sup> P. Lawyer,<sup>2</sup> P. Tukei,<sup>5</sup> R. Trotter,<sup>1</sup> J.  
White,<sup>1</sup> B. Hall,<sup>1</sup> M. Kiley,<sup>3</sup> D. Silverstein,<sup>4</sup> R. Zimmerman,<sup>4</sup> B.  
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Medical Research Institute.

Marburg virus, a rare, highly pathogenic virus, was isolated from a fatal hemorrhagic fever virus case at Nairobi Hospital Kenya. Virus was isolated in cell culture, identified by an indirect immunofluorescent antibody assay using Marburg virus disease convalescent sera and Marburg virus reactive monoclonal antibody and electronmicroscopy. The isolate produced typical Marburg virus disease when inoculated into rhesus macaques. The histopathological lesions in infected monkey tissues were similar to the lesions observed in autopsy specimens. The case history suggested that the fatal exposure occurred at Kitum cave on Mt. Elgon located along the Uganda/Kenya border. Sentinel animal (monkeys and guinea pigs) studies, field collections of animals and biting arthropods, and serological surveys of cave dwelling human and livestock populations were conducted to define Marburg virus activity in the Mt. Elgon cave environment. The results of the ecological and epidemiology studies at Kitum cave will be discussed.

- 140 EPIDEMIOLOGY OF DENGUE VIRUS SEROTYPES 1 AND 2 AS REVEALED BY PRIMER-EXTENSION SEQUENCING. Rebeca Rico-Hesse. Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, CT.

Primer-extension sequencing has recently helped to elucidate wild poliovirus transmission patterns and dynamics, thus yielding information important in controlling disease. This method has now been used to study the epidemiology of dengue viruses of serotypes 1 and 2, isolated over a 43 year span. A portion of the NS1 region of the dengue genome showed fixation of mutation rates of around 7% across isolates from different geographic areas. The majority of these mutations occurred in the 3rd position of the codon. Other NS1 and capsid sequences showed an equivalent rate of mutation, but the sites were not limited to the 3rd position, and many amino acid changes occurred across strains. Nucleotide sequences from the NS1 region of the genome of all isolates were compared to each other by a computer program, and a dendrogram of genetic relationships was generated. Analysis of <5% of the genome revealed that the evolutionary patterns of dengue viruses of serotypes 1 and 2 are different, as are the transmission pathways of these viruses across the world. The relationships thus established follow those defined previously by oligonucleotide fingerprinting although much broader relationships were revealed. Thus, limited genomic sequencing for the determination of transmission pathways: 1) is applicable to RNA viruses other than polio, even though mutation rates were lower, 2) gives results that are easy to interpret and are directly comparable across serotypes as well as within serotypes, and 3) has allowed the definition of broader dengue virus genetic relationships than was possible with oligonucleotide fingerprinting.

- 141 ASSOCIATION OF ENCEPHALOPATHY AND HEMORRHAGE WITH BIOPSY PROVEN HEPATIC NECROSIS IN DENGUE TYPE-3 INFECTION IN THAILAND, 1987.

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In 1987, Thailand experienced the largest recorded dengue (DEN) epidemic in its history. We found that DEN-3 was, for the first time since 1963, the most common isolate in Bangkok. By mid-epidemic, many clinicians had noted the occurrence of DEN with "unusual" manifestations. We participated in a regional surveillance network to prospectively enter DEN patients into a data base. "Unusual" cases were defined as those with either encephalopathy (stupor or coma), admission serum alanine aminotransferase >200 U/L or admission serum creatinine >250 uM/L. Thirty nine "unusual" and 180 "usual" confirmed DEN infections were analyzed. Manifestations of vasculopathy and plasma leakage, including shock, were equally distributed between both groups but hemorrhage was significantly more common among "unusual" cases. The presence of GI bleeding, Babinski reflex, jaundice, oliguria and the degree of encephalopathy was associated with the admission serum ALT by linear regression. Thirteen patients died (all "unusual"). Nine post mortem liver biopsies were obtained and all showed moderate to severe necrosis in a midzonal, centrilobular or sub-massive distribution. Thirty DEN isolates were made. DEN-3 accounted for 40% and was the only isolate made from unusual cases (2 cases, both had midzonal necrosis). Acute liver failure is part of the spectrum of DEN infection. Two DEN-3 viruses were isolated from patients with yellow-like illnesses. Increased recognition of this syndrome in 1987 may have been due to its association with the DEN-3 strain(s) circulating.

D: ARBOVIROLOGY - EPIDEMIOLOGY AND PATHOGENESIS

- 142 Epidemic Dengue 2 in the Republic of Palau. D. J. Gubler,\*  
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The last known epidemic of dengue in the Palau Islands occurred during the 1943-1944 pandemic that affected most Pacific islands. In early 1988, an increase in incidence of viral syndrome was noted on the main island of Koror. In late March, an adult female with a viral prodrome died of an illness compatible with dengue hemorrhagic fever. Subsequently two other fatal cases were reported. Seroepidemiologic studies using the IgM-capture ELISA confirmed transmission on three islands, Koror, Peleliu, and Kayangel. The overall attack rate on Koror was 48% and on Peleliu, 43%. It was not possible to accurately measure the rate on Kayangel. In Koror, with a population of approximately 9,400, it is estimated that approximately 4,500 dengue infections occurred. Dengue 2 virus was isolated from 20 patients, including one fatal case. Preliminary genetic analysis of the virus strain shows that it is closely related to the Philippine topotype. Mosquito surveys revealed that both Aedes aegypti and Aedes albopictus were present on Koror. These species had never been reported in Palau prior to this report. Both species had wide spread distribution, but indices were highest in the port area, suggesting that the mosquitoes had been introduced via the shipping industry. A review of the Ports Authority shipping log revealed many ships calling in Palau from countries where both species occur, including the Philippines and Indonesia.

- 143 MONOCYTE-INFECTIVITY AS A VIRULENCE MARKER FOR DENGUE-2 VIRUS  
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at Berkeley, Berkeley, CA and The National Institutes of Health,  
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Seventy one strains of dengue-2 viral isolates from eight geographic locations where dengue infections are endemic or sporadic have been tested for their infectivity in human monocytes. Of these 71 isolates, 32 were associated with clinical symptoms as dengue fever (DF) while the other 39 were associated with dengue hemorrhagic fever of various grades. The infectivity test was performed using freshly isolated human monocytes in the presence and the absence of dengue-2 polyclonal antibodies at the optimal enhancing concentration. The infection rates were measured by examining and quantitating infected cells as detected by the indirect fluorescent antibody (FA) technique. In addition, virus production from the four day monocyte cultures was measured using the plaque assay on BHK-21 cell monolayers. Statistical analyses of our data indicated a correlation between the probability of a viral isolate to cause severe illness (DHF/DSS) and the monocyte-infection rate as measured by the viral yield in the presence of enhancing antibodies and the FA staining pattern of infected monocytes. We also tested 24 viral isolates associated with DHF/DSS from locations where DHF/DSS is endemic for an association between the different grades of DHF severity and the rate of viral infection in monocytes. There was no statistically significant association between the different grades of severity and the infection outcome. These findings suggest that virulence of dengue-2 virus as defined by the ability of the virus to cause DHF/DSS can be identified by the viral infectivity in human monocytes. However, variation in the severity of DHF may be associated with other factor.

E: FILARIASIS - BIOCHEMISTRY AND MOLECULAR BIOLOGY

- 144 SYNTHETIC AND NATURALLY OCURRING RETINOIDS INHIBIT THIRD- TO FOURTH-STAGE LARVAL DEVELOPMENT BY ONCHOCERCA LIENALIS IN VITRO.  
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The discovery of retinoid binding proteins in Onchocerca volvulus which are distinct in their physicochemical properties from corresponding host proteins has suggested retinoid metabolism as a site of chemotherapeutic attack in this parasite. With this as a rationale, a series of synthetic retinoids was screened for the ability to inhibit the third-to fourth-stage larval molt by O. lienalis in vitro. Of the 14 retinoids tested, eight gave significant inhibition of the molt at a concentration of 10-5 g/ml or less. Probit analysis of dose-response data collected for these active compounds indicated values for ED50 in the range of 3.7 - 17.1  $\mu$ M. In general, the most active of these N-substituted retinamides were those with small alkyl or monohydroxy alkyl substituents. The most active molecule in this class was all-trans-N-(2-hydroxyethyl)retinamide with an ED50 of 3.7  $\mu$ M. Both the all-trans and 13-cis isomers of the alkyl substituted derivatives were active, the all-trans-N-hydroxyethyl derivative being approximately 5 times as potent as the corresponding 13-cis isomer. The N-2,3 dihydroxypropyl derivative, two derivatives with aromatic side chains and three N-(retinoyl)amino acids were inactive by the criteria set in the initial screening. There was no strict correlation between growth regulating activity against O. lienalis and binding affinity for a retinol binding protein from Dirofilaria immitis. These findings argue for further study of retinoids as potential antifilarial agents. Supported by the UNDP/World Bank/WHO, the E. M. Clark Foundation and NIH grants EY-03984, EY-06616 and CA-34968.

- 145 ISOLATION AND PRELIMINARY CHARACTERIZATION OF GLYCOLIPID AND GLYCOLIPID OLIGOSACCHARIDE RESIDUES OF ADULT ONCHOCERCA GIBSONI.  
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The first objective of the research reported here was to obtain quality adult worm tissue as free as practical of host tissue contaminants and then to extract and characterize the parasite glycolipids and glycolipid oligosaccharides. Frozen connective tissue nodules containing Onchocerca gibsoni adults were obtained directly from Australia and sectioned while still frozen at -70°C into ~4 mm slabs. Frozen sections were cleaned of any adhering kerf, thawed, and the worm segments rapidly removed into ice cold Hanks' Balanced Salt Solution containing no glucose or phenol red. The worm segments were rinsed twice in excess Hanks', centrifuged at 11,000 xg for 2 min at 4°C and stored in liquid nitrogen as 0.25 g pellets. The pellets were extracted with chloroform/methanol and the extract used either directly to identify and isolate glycolipids or processed to obtain free oligosaccharides. Adult O. gibsoni were found to possess both neutral and charged glycolipids, some of which were positive for sialic acid. Neutral glycolipids comigrating on thin layer chromatograms with cerebroside and ceramide dihexoside standards were observed along with others possessing longer carbohydrate chains. High performance liquid chromatography (normal and reverse phase) was used to identify and isolate individual oligosaccharides and establish oligosaccharide profiles of worm segments and host tissue. These results should provide insight into the complexity of worm carbohydrate antigens and avenues to study the immunogenicity of these antigens. (Supported by Edna McConnell Clark Foundation Grant No. 13087.)

IN VIVO AND IN VITRO KILLING OF BRUGIA MALAYI LARVAE AND MICROFILARIAE BY CGP 20 376 (CIBA-GEIGY LIMITED). J. A. Yates, K. L. Hellner\*, and G. I. Higashi, University of Michigan, School of Public Health, Department of Epidemiology, Ann Arbor, MI.

A new macrofilaricidal drug, CGP 20 376, was evaluated for its capacity to kill subperiodic *B. malayi* larvae in vivo in jirds and also at various concentrations in vitro. In vitro studies were performed in 24-well culture plates to evaluate the effects of drug concentrations ranging from 1000 ug/ml to 0.01 ug/ml on infective stage larvae (L3) and microfilariae (mf). Culture wells containing 500 mf each, or 20 L3 each, were dosed with 10-fold dilutions of CGP 20 376 suspended in dimethyl sulfoxide (DMSO) and medium. MF and L3 were killed by CGP 20 376 in vitro in the absence of serum factors. MF were killed within 2 hours at drug concentrations of 1000- and 100 ug/ml. Killing reached 100 percent by 24 hours with 0.1 ug/ml of the drug, while complete killing required 35 hours at the lowest concentration studied, 0.01 ug/ml. Microfilariae in medium only or in medium with DMSO were more than 99 percent viable after 35 hours in culture. For L3, drug concentrations of 1000- and 100 ug/ml killed 100 percent of the larvae by 2.5 hours in culture and by 15 hours with 10 ug/ml. In 1 ug/ml 50 percent were dead by 20 hours and 90 percent were killed by 25 hours. However, 6 larvae (0.8 percent) remained alive and sluggishly motile for 165 hours. The effect of CGP 20 376 on developing larvae in *M. unguiculatus* was evaluated in 3 groups of age-matched, inbred, male jirds (14 animals per group). These studies indicated that a single dose of 25 mg/kg of CGP 20 376 was more than 99% effective against fourth stage larvae in vivo.

HYDROGEN PEROXIDE IS TOXIC FOR ONCHOCERCA MICROFILARIAE

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Eosinophilia is associated with most helminth infections; and eosinophils adhere to and/or kill many helminths in vitro. Eosinophils surround *Onchocerca cervicalis* microfilariae (mf) in infected horse skins apparently without toxic results despite their ability to release granule proteins and oxidants-principally superoxide radicals and hydrogen peroxide. Anti-oxidant enzymes, including superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-px), can potentially protect parasites from oxidant-mediated host attack. The effect of reactive oxygen species on mf in vitro was investigated and the level of endogenous anti-oxidant enzymes determined.

Mf cytotoxicity resulted from low levels of hydrogen peroxide added directly, hydrogen peroxide generated by glucose-glucose oxidase, and hydrogen peroxide and superoxide radicals generated by xanthine-xanthine oxidase. Catalase, but not inactivated catalase or SOD, protected mf from oxidant-mediated cytotoxicity. Desferal, which chelates Fe and prevents the formation of hydroxyl radicals generated through the reaction of ferrous ions with hydrogen peroxide, does not inhibit toxicity in either hydrogen peroxide system. Preliminary results indicate that mannitol, a hydroxyl radical scavenger, also does not protect in the xanthine-xanthine oxidase system. These data indicate that mf are sensitive to hydrogen peroxide, but not superoxide or hydroxyl radicals.

Anti-oxidant enzyme levels are similar in mf and adult worms of *O. cervicalis*. The adult worm homogenate contains 27.6 U/mg SOD, 1 mU/mg catalase and 6.8 mU/mg GSH-px, while mf contain 29.5 U/mg SOD, 4 mU/mg catalase, and 9 mU/mg GSH-px. These SOD levels are high, while the hydrogen peroxide scavenging enzyme levels are low-at the limits of detection. These results may explain the mf sensitivity to hydrogen peroxide toxicity and the ability of mf to resist superoxide radicals. [Supported by NIH grants EY05757, EY06462 and EY07542].

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### THE CHARACTERIZATION OF A HEAT SHOCK PROTEIN 70 (HSP70) GENE OF *BRUGIA MALAYI*.

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Although most people living in an area endemic for lymphatic filariasis are exposed to third stage larvae, few exhibit clinical symptoms. The clinical spectrum ranges from seemingly anergic asymptomatic microfilaremic patients to apparently immune amicrofilaremic patients. Using sera from individuals living in endemic areas to screen *Onchocerca volvulus* cDNA libraries, we have isolated several interesting clones. One clone, OvG15, which is primarily recognized by amicrofilaremic individuals (endemic normals and patients exhibiting chronic symptoms) is homologous to *Xenopus laevis* heat shock 70 protein (84.7% identity and 97% conservation) as well as to rat heat shock cognate protein (85.4% identity and 97% conservation). Using this clone, genomic HSP70 clones were isolated from a *B. malayi* library. The sequences of the HSP70's from the two species were compared. An attempt is being made to characterize the promoter region of the *B. malayi* gene to determine whether it contains sequences associated with the induction of heat shock genes by various stimuli. The heat shock response might be a developmental switch and hence has great biological significance. In addition, the recognition of HSP70 primarily by amicrofilaremic individuals implies a possible role in protection.

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### Characterization of 2 *Brugia malayi* repeat families

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We have previously reported that restriction fragment length polymorphisms can be useful in distinguishing between closely related parasite species. In order to find DNA probes which could identify *B. malayi* subspecies, we sought and have found interspersed repeat families. Interspersed repeats, in contrast to single-copy or tandemly repeated sequences, produce complex patterns on Southern blots and scan a large proportion of the genome, so that even minor differences can be readily visualized.

To find such families, we constructed several *B. malayi* libraries by cloning Mbo I digests of genomic DNA into the Bam HI sites of M13mp18 and lambda Charon 27. Such libraries exclude the major (Hha I) repeat family of *B. malayi*, as this family is tandemly arrayed and lacks an internal Mbo I site. We initially screened the M13 library with nick translated *B. malayi* genomic DNA, and isolated several clones. Subsequent sequence analyses revealed that they belonged to distinct repeat families, the Bm 4 family and the Bm 11 family, both of which appear to be moderately repetitive, interspersed, and unrelated to each other or to the Hha I repeat family. Members of the Bm 11 repeat family were also isolated from the Charon 27 library.

When these repeats were used to probe Southern blots of DNA of *B. malayi* individuals from North American laboratory stocks, there was no variation in the pattern of bands seen. This suggests that the repeats are stable and should be ideal candidates for taxonomic analyses.

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## MOLECULAR GENETICS OF FILARIAL MYOSIN.

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We have previously reported the construction of a *Brugia malayi* expression library in  $\lambda$ gt11 and the identification of an antigen strongly recognized by a single patient with Tropical Pulmonary Eosinophilia as *B. malayi* myosin. Sequence analysis of this clone and comparison to the body wall myosin, *unc-54*, of *Caenorhabditis elegans* revealed 98% amino acid conservation (80% identity) but only a 61% identity at the nucleotide level, due mostly to a biased codon usage in the *B. malayi* gene. The comparison also localized our clone to a short region within the light meromyosin tail. We have since determined that reactivity to this clone with antibodies of the IgG, but not IgE class, can be detected in sera from many patients with various clinical manifestations of filariasis. We have used this clone at the protein level to raise antisera to analyze the native myosin protein, and as a DNA probe to isolate genomic fragments encompassing the entire gene. By Southern blot analysis, the gene unexpectedly appears to be associated with the *B. malayi* major tandem repeat. Sequence analysis is underway to verify this, as well as to determine the relationship of the repeat to the *B. malayi* myosin coding sequence. In addition, we have used the probe to identify an *Onchocerca volvulus* clone primarily recognized by asymptomatic microfilaremic individuals, and are sequencing this clone to clarify the evolutionary relationship of the two filarial species.

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SEQUENCE AND GENOMIC ORGANIZATION OF THE MAJOR SPERM PROTEIN GENES FROM *ONCHOCERCA VOLVULUS*

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Nematode spermatozoa, unlike their mammalian counterparts, are rounded, nonflagellated cells that exhibit amoeboid locomotion. Nematode sperm contain an abundant protein, the major sperm protein (MSP), which comprises more than 15% of the protein in the cell. MSP assembles into 2-3nM filaments in the spermatozoan pseudopod where it is presumed to play a role in motility. The high degree to which MSP coding sequences have been conserved through nematode evolution has allowed us to use *Ascaris* MSP cDNA as a probe to identify and isolate recombinant clones from an *O. volvulus* genomic lambda gt11 library. Two clones, MSP 1A (765 bp) and MSP 2B (1765 bp), were characterized by restriction endonuclease mapping and sequences analysis. Sequence analysis showed that both MSP genomic clones contain two protein coding regions of 99 and 281 bp separated by an intervening sequence of 152 bp. MSP 1A and MSP 2B were 96% similar in nucleotide sequence over the protein coding regions, but only 79% similar when the intervening sequences were compared. The nucleotide and presumptive amino acid sequences of the MSP 1A and MSP 2B were compared with those of *Ascaris* and *Caenorhabditis* MSP cDNAs. Over the protein coding region of the genes, the *O. volvulus* MSP nucleotide sequences were 83% and 79% similar to *Ascaris* and *Caenorhabditis*, respectively. When the presumed amino acid sequences were compared, *O. volvulus* MSP was 84% similar to *Ascaris* and *Caenorhabditis*. *O. volvulus* MSP appears to contain only 2 to 3 MSP genes in its genome. This low number of MSP genes is in contrast to the over 60 MSP genes found in *Caenorhabditis*, but is similar to the single MSP gene found in the genome of *Ascaris*. Data on the genomic organization of MSP genes in a number of free-living and parasitic nematode species is also presented.

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PURIFICATION OF GLYCEROL-3-PHOSPHATE OXIDASE FROM THE MITOCHONDRIA OF *TRYPANOSOMA BRUCEI*

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The bloodstream forms of African trypanosomes are completely dependent on glycolysis for their energy supply and utilize a unique shuttle, which includes a terminal oxidase, to reoxidize the glycolytically produced NADH. This terminal oxidase, which is located in the mitochondrial membrane, is cytochrome independent, and not inhibited by classical inhibitors of the respiratory chain. This enzyme complex consists of two components: a flavin-linked glycerol-3-phosphate dehydrogenase and a glycerol-3-phosphate oxidase (GPO) which are probably linked via ubiquinol. The oxidase component of the enzyme is absent in the mammalian host and is specifically inhibited by salicylhydroxamic acid (SHAM).

We describe here the purification of the GPO from the bloodstream form of African trypanosomes. Mitochondria from *Trypanosoma brucei* bloodstream trypomastigotes were treated with 7.5 mM lauryl maltoside to release the GPO from the mitochondrial membrane. Then 1.5 mg (total protein) of solubilized mitochondria was applied to a Pharmacia Mono Q HR 5/5 anion exchange column and chromatography was performed on a Pharmacia FPLC system. All fractions were measured for enzyme activity using a Instech 125/05 oxygen electrode in 0.5 ml buffer containing 40 mM Tris (pH 8.0) and 3 mg/ml bovine serum albumin with 10 mM glycerol-3-phosphate or 0.6 mM of a ubiquinol analog as substrate. A 180 fold enrichment for the GPO and a 30 fold for the ubiquinol oxidase was obtained. To confirm the oxidase activity, 0.5 mM SHAM was used which inhibited the oxidase activity 100%. Eighty-five percent of the protein was recovered from the column. The purity of the enzyme is currently being followed by increasing specific activity and SDS-PAGE patterns.

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3-METHOXYPHENYLACETIC ACID, AN INHIBITOR OF FUMARATE REDUCTASE IN *Trypanosoma brucei*, STIMULATES H<sub>2</sub>O<sub>2</sub> PRODUCTION IN INTACT CELLS.

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Mitochondria from *Trypanosoma brucei* procyclic trypomastigotes contain the enzyme NADH-fumarate reductase, which catalyzes the reduction of fumarate to succinate (the reversal of the reaction catalyzed by succinate dehydrogenase in the Krebs cycle). This enzyme may generate O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> in a two to one ratio when incubated with NADH in the absence of fumarate (Turrens, J.F., Molec. Biochem. Parasitol. 25: 55-60, 1987). Merck, Sharp and Dohme Laboratories kindly provided several drugs known to inhibit the fumarate reductase isolated from a different organism (*Haemonchus contortus*). These drugs may be useful tools for determining the role of fumarate reductase in the metabolism of mitochondria from *T. brucei* procyclic trypomastigotes. One of these compounds (3-methoxyphenylacetic acid [3-MPA]) inhibited the activity of the NADH-fumarate reductase with a K<sub>i</sub> = 10 uM. When added to intact cells it inhibited respiration (K<sub>i</sub> = 9 uM) and it also inhibited cell growth with a very similar K<sub>i</sub> (10 uM), suggesting that both events may be related. In addition, when added to a suspension of *T. brucei* procyclic trypomastigotes, 3-MPA stimulated H<sub>2</sub>O<sub>2</sub> production (determined as the rate of Compound I formation with cytochrome c peroxidase) in a dose dependent manner. These results suggest that 3-MPA may affect the activity of the NADH-dehydrogenase of *T. brucei* stimulating its capacity to transfer electrons directly to oxygen, generating H<sub>2</sub>O<sub>2</sub>.

This work was supported by a Grant from the UNDP/World Bank/WHO (ID# 870264), and by a Research Grant from Merck, Sharp and Dohme Laboratories.

F: BIOCHEMISTRY AND MOLECULAR BIOLOGY OF KINETOPLASTIDA

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BIOCHEMISTRY OF PENTOSTAM-RESISTANT LEISHMANIA. J.D. Berman,\* M. King, N. Edwards, and M. Crogl. Walter Reed Army Institute of Research, Washington, DC.

Leishmaniasis clinically resistant to treatment with Pentostam (Sb) constitutes a significant percentage of infections in many endemic regions. To study Sb resistance, we generated Leishmania mexicana amazonensis resistant to Sb (WR669R) by exposure of promastigotes of a sensitive clone (WR669) to increasing Sb concentrations. We then generated amastigotes of WR669R and WR669 by infection of J774 macrophages with the respective promastigotes, and by disruption of the infected macrophages. The concentration of Sb needed to kill 50% of organisms in physiologic salt solutions exposed to drug for 3 hours was: WR669R amastigotes (7,000 $\mu$ g/ml); WR669R promastigotes (10,000 $\mu$ g/ml); WR669 amastigotes (150 $\mu$ g/ml); WR669 promastigotes (200 $\mu$ g/ml). This data shows that the two Leishmania forms (amastigotes and promastigotes) have comparable Sb susceptibility when tested under identical conditions. The uptake of  $^{125}$ Sb-Pentostam over 1 hr by WR669R amastigotes was comparable to that of WR669 amastigotes. This indicates that the resistance of WR669R is not caused by altered initial accumulation of drug. Glycolysis and fatty acid oxidation in WR669 amastigotes, but not in WR669R amastigotes, was inhibited by Pentostam, which demonstrates a correlation between Sb-induced killing and inhibition of bioenergetic pathways in amastigotes.

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METACYCLIC STAGE-SPECIFIC cDNA CLONES OF L. MAJOR AND T. CRUZI .

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It has previously been demonstrated that the hemoflagellated protozoa Leishmania major and Trypanosoma cruzi undergo a developmental change (metacyclogenesis) immediately before leaving the insect vector which pre-adapts them for survival in the mammalian host. This transformation from non-infective to infective stage can be duplicated in vitro by allowing insect forms (promastigotes or epimastigotes) to reach stationary phase. We are attempting to study gene regulation during metacyclogenesis of these two parasites by initially identifying genes which are preferentially expressed in the metacyclic stages. Toward this end we have identified several cDNA clones which are metacyclic stage specific. L. major clone pLM3.16 (250bp) hybridizes to a transcript of 900bp and has a 75% sequence homology in a 60 amino acid overlap to the  $\beta$ -chain of E. coli RNA polymerase. T. cruzi clone pTC43.1 (450bp) hybridizes to three transcripts of 3.0kb, 2.4kb and 2.0kb. Restriction enzyme and Southern blot analysis suggests that pTC43.1 is a member of a small multigene family. Additional data from Southern blots of T. cruzi and L. major genomic DNA demonstrates that there is restriction site conservation between the two species within this gene family. The deduced amino acid sequence from this cDNA clone demonstrates a 42% homology in a 35 amino acid overlap with the proto-oncogene c-fos. The above findings suggest that the leishmania and T. cruzi genes we have cloned encode proteins involved in gene regulation.

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- 156 MOLECULAR KARYOTYPE ANALYSIS OF LEISHMANIA BRAZILIENSIS PANAMENSIS.  
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We have used molecular karyotyping to assess the degree of genomic variability occurring among members of Leishmania species. Eleven paired isolates of L. b. panamensis were collected from 5 patients with primary and recurrent cutaneous leishmaniasis contracted in the Pacific Coast region of Colombia. 9/11 isolates recovered from all 5 patients belonged to the same zymodeme (23); 2 additional isolates belonging to another zymodeme (22) were also recovered from one patient. Molecular karyotypes of isolates had 22 resolved bands, characterized by transverse alternating field electrophoresis (TAFE) and by hexagonal pulsed field gradient gel electrophoresis (HE-PFG). Each patient was infected with one or more karyotypically unique isolates. In 4/5 patients, both paired isolates had identical karyotypes; in 1/5, they differed distinctly. We conclude that a high degree of chromosomal variability occurs within L. b. panamensis, even within the same zymodeme from a discrete geographic area.

All isolates had chromosomes sized ~ 275, 400, 500, 850, 900 and 1050 kb, estimated by comparison with relative mobilities of multimers of bacteriophage lambda. These chromosomes characterized the L. b. panamensis karyotype such that members of this subspecies could be readily identified. Hybridization of Southern blots of TAFE and HE-PFG gels with DNA purified from single chromosome bands, and with cloned gene probes for Hsp-70 (70 kd heat shock protein of L. major) and  $\alpha$ ,  $\beta$ -tubulin (from T. brucei) revealed that L. b. panamensis size-homologous chromosomes also shared a high level of sequence homology.

- 157 INTERFERON-GAMMA (IFN $\gamma$ ) INDUCES ALKALINIZATION OF LEISHMANIA CONTAINING PHAGOLYSOSOMES (PL). D.J. Wyler<sup>\*</sup>, M.V. Callahan, and R.B. Mikkelsen. New England Medical Center Hospitals, Inc., and Tufts University School of Medicine, Boston, MA.

Previous studies of others have shown that amastigote metabolism is optimum at acidic pH. Leishmania are susceptible to the antimicrobial effects that result from macrophage activation by IFN $\gamma$ . We therefore investigated whether the mechanism of IFN $\gamma$ -mediated antileishmanial effects involves alterations in the pH of PL. L. mexicana amazonensis-containing PL were loaded with the pH sensitive dye fluorescein-dextran and were studied by digitized video-intensified fluorescence microscopy. Using published methods, we established that the PL pH is 4.0-4.5, but rises rapidly to as high as pH 8 following infusion of IFN $\gamma$ . Neither IFN $\beta$  nor selected other cytokines had this effect. PL containing a strain of L. m. amazonensis resistant to IFN $\gamma$  antimicrobial effects also was alkalinized in this manner. On the other hand, amastigotes of this resistant strain incorporated uridine over a wide range of pH conditions, whereas the IFN $\gamma$  sensitive strains did so at pH<6. We conclude that the antileishmanial effects of IFN $\gamma$  results from PL alkalinization which creates an inhospitable environment for the acidophilic amastigote.

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- 158 EXACERBATION OF *Leishmania major* INFECTION IN MICE TREATED WITH MONOCLONAL ANTI-INTERFERON $\gamma$ . M. Belosevic\*, D.S. Finbloom, P.H. Van der Meide and C.A. Nacy. Univ. of Alberta, Canada; Primate Center TNO, The Netherlands; and Walter Reed Army Inst. of Res., Washington, DC.

Interferon $\gamma$  (IFN $\gamma$ ) is a pluripotent lymphokine that participates both in the induction of immune reactions (upregulation of Ia molecules on antigen presenting cells) and in the effector arm of immunity (activation of macrophages for tumoricidal and antimicrobial activities). That IFN $\gamma$  activates macrophages to kill *Leishmania* is documented in many *in vitro* studies, and a correlation of susceptibility to certain *Leishmania* with decreased production of IFN $\gamma$  is reported. C3H mice develop small cutaneous lesions following the inoculation of *L. major* into footpads: these lesions heal within 12 wks with no evidence of systemic spread of the parasite. We examined the course of infection with *L. major* in naturally resistant C3H/HeN mice after i.p. administration of murine monoclonal antibody (Ab) prepared against recombinant IFN $\gamma$ . Administration of 1 mg/mouse/wk Ab markedly altered the course of disease: cutaneous lesions (lesion size 3.2 mm at 8 wks vs 0.3 mm in mice treated with an irrelevant Ab of the same isotype) and numerous parasites (numbers greater than untreated susceptible BALB/c mice) in foot, regional lymph node, liver, and spleen were noted. The minimal dose of Ab to effect this change was 0.5 mg/wk/mouse; 0.1 mg/wk/mouse induced lesions of intermediate size (1.5 mm at wk 8). However, a single treatment of 1 mg/mouse at the initiation of infection (Ab has half-life of 30 days) was sufficient to induce lesions similar to those of mice treated with 0.5 mg/wk/mouse. IFN $\gamma$  present at the initiation of infection by *L. major* is clearly necessary for successful resolution of cutaneous disease and containment of metastatic spread of the parasite in the naturally resistant C3H/HeN mouse. Whether Ab to IFN $\gamma$  can also abrogate an effective immune response in the resolving cutaneous lesion is the subject of ongoing studies.

- 159 RESPIRATORY METABOLISM OF METACYCLIC AND EPIMASTIGOTE STAGES OF AFRICAN TRYPANOSOMES.  
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The ability to cultivate epimastigotes and metacyclic trypomastigotes of *T. congolense* and *T. brucei* in sufficient quantities for biochemical analysis has enabled us to study the respiratory metabolism of these cells. The data suggest that while epimastigotes are biochemically similar to procyclic trypomastigotes, metacyclics represent a stable metabolic intermediate between the procyclic/epimastigote stages and the bloodstream trypomastigote. Epimastigotes, like procyclic cells, preferentially utilize proline as an energy source and have a fully functional mitochondrial cytochrome electron transport system accounting for the majority of terminal oxidase activity. Metacyclics have reduced cytochrome function (the trypanosome alternative oxidase being the primary terminal oxidase) with glucose as the preferred substrate. These metabolic data contrast markedly to what is seen ultrastructurally; metacyclic mitochondria still retain the heavily cristate appearance of procyclic and epimastigote forms. Studies are underway to further characterize the metabolism of these vector stages.

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160 FURTHER STUDIES ON EXTRACELLULAR DEVELOPMENT OF PLASMODIUM FALCIPARUM  
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Intraerythrocytic malaria parasites are among the many species of protozoa that are obligate intracellular parasites. Why should a eucaryotic organism having its own protein-synthesizing machinery depend on a living host cell for its development, and what is the nature of this dependence? One approach to answering these questions has been provided by the observation that merozoites of *P. falciparum* show an initial development into small ring-like forms in a red cell extract medium supplemented with ATP and pyruvate (W. Trager & H.N. Lanners, 1984, J. Protozool. 31,562-567). Merozoites for these experiments were prepared by incubating a concentrated suspension of schizonts for 1½ hours and then removing the schizonts by low speed centrifugation. The supernatant contained free merozoites (50 to 200 x 10<sup>6</sup> per ml) and was used to inoculate experimental media at the rate of 0.1 ml to 1 ml medium. From 0.5 to 5% of the merozoites were invasive. We have now found that ADP can replace ATP, since in the presence of pyruvate or phosphoenol pyruvate ATP is formed in the red cell extract medium. We have compared the fine structure of rings that have developed extracellularly from merozoites with that of rings that have developed intracellularly and have then been lysed out from the red cell with hemolytic antiserum and complement. Very similar forms are seen, the main differences being that the forms freshly removed from the cell have more ribosomes and are surrounded by a parasitophorous membrane. The viability of the small forms that develop extracellularly is further indicated by their uptake of rhodamine 123 as seen by fluorescence microscopy.

161 CHARACTERIZATION OF THE 5.8S RIBOSOMAL RNA CODING  
DOMAINS FROM *PLASMODIUM FALCIPARUM*.  
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RNA and dideoxy sequence analyses in our laboratory have previously demonstrated that the 5.8S rRNA component, expressed during the parasite's asexual erythrocytic life cycle, is composed of a single homogeneous population of molecules which are 157 nucleotides in length. Reports from other laboratories have shown that the plasmodial rRNA transcription units from which the 5.8S rRNA is derived are heterogeneous in spacer and coding regions. In order to determine the number and diversity of potentially active large rRNA gene classes, we have undertaken restriction endonuclease and dideoxy sequence analyses of the 5.8S rRNA locus present in genomic or cloned DNA. Southern blot analyses indicate that there are approximately 5 rRNA gene classes (Class I-V) present in the *P. falciparum* genome. Four of these have been cloned in EMBL-3 phage lambda. Dideoxy sequence analyses of the 5.8S coding region contained within these recombinants indicate that only Class II transcription units are expressed during the erythrocytic life cycle. The other classes contain small insertions, point mutations or truncations within the 5.8S coding domain. Northern blots of total erythrocytic stage RNA hybridized to probes specific for 4 of the 5 classes detected only Class II transcripts. Copy number analyses indicate that the Class II rRNA genes are present in a higher proportion in the genome. It is postulated that the variant classes (Class I, III, IV) may represent low copy rRNA pseudogenes. In addition, restriction endonuclease digestion analyses of the lambda recombinants indicate that some of the *P. falciparum* rRNA transcription units are linked in the genome.

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152      ROLE OF THE PLASMODIUM FALCIPARUM MEROZOITE SURFACE ANTIGEN  
(PF 190-200) IN ERYTHROCYTE INVASION

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The most prominent and well characterized of the Plasmodium falciparum merozoite surface antigens is a protein of m.w. 200,000. The m.w. of the protein varies in different strains and has been reported to range from 190,000 to 205,000. The complete sequence of Pf 200 from seven different isolates of P. falciparum has been reported. Although the protein displays regions of polymorphism extensive sequence domains of both the N-terminal end and the C-terminal end are conserved between isolates. We have previously demonstrated that a monoclonal antibody directed against the C-terminal disulfide bonded end of the antigen, partially blocks merozoite invasion into erythrocytes (Pirson and Perkins, J. Immunol. 134:1946, 1985). To investigate the role of Pf 200 in merozoite invasion we examined the binding properties of the isolated antigen to human erythrocytes. Low affinity binding between the erythrocyte surface and Pf 200 was detected. Pf 200 also bound to erythrocytes from Saimiri monkey, which are invaded by P. falciparum. However, it did not bind to Rhesus, mouse or rabbit erythrocytes, species which are not infected with the FCR-3 strain of P. falciparum. These results suggest that the interaction between Pf 200 and human erythrocytes is low affinity but highly specific for the correct host cell. Pf 200 may be the merozoite surface protein responsible for recognition of the host erythrocyte.

163      ROLE OF RHOPTRIES IN P. FALCIPARUM MEROZOITE INVASION OF ERYTHROCYTES

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During erythrocyte reinvasion by P. falciparum merozoites, immunofluorescence demonstrates that the 110 kd rhoptry protein is discharged from the merozoite and is secreted into the erythrocyte membrane. In the newly reinvaded erythrocyte, the protein is located in the plasma membrane and cytoplasm (Sam-Yellowe, T. et al., J. Cell Biol. 106:1507-1513). These results suggest that the 110 kd rhoptry protein is inserted into the membrane of the host erythrocyte at the time of merozoite invasion. Immunoelectron microscopic studies show that the 110 kd protein is located in the matrix of the organelle and on membranous whorls secreted from the merozoite. Further characterization of the protein properties by immunoblotting and immunoprecipitation show that MAb recognition of the epitope is dependent on disulfide bond linkage. Furthermore, MAb 189 coprecipitates other rhoptry proteins of 140 kd and 130 kd during immunoprecipitation suggesting that most of the rhoptry proteins exist in a complex within the organelle. We are currently investigating the biochemical interaction of the rhoptry protein with the proteins of the host membrane. This may involve binding to spectrin, band 3, band 4.1 or minor host proteins.

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- 164      ACTIVATION OF MONOCYTES AND PLATELETS BY MONOCLONAL ANTIBODIES OR  
*Plasmodium falciparum*-INFECTED ERYTHROCYTES BINDING TO THE CD36  
SURFACE RECEPTOR *in vitro*.

Christian F. Ockenhouse,\* Cathleen Magowan and Jeffrey D. Chulay. Dept of Immunology, Walter Reed Army Institute of Research, Washington, DC.

The leukocyte differentiation antigen CD36 (cluster determinant 36), recognized by monoclonal antibodies (MAbs) OKM5 and OKM8 and found on human monocytes and endothelial cells, has been implicated as a sequestration receptor for erythrocytes infected with the human malaria parasite *Plasmodium falciparum*. CD36 is also expressed on platelets and appears to be identical to platelet GPIV. We investigated receptor activation of monocytes and platelets by anti-CD36 MAbs and by malaria-infected erythrocytes (IRBC). Incubation of human monocytes with anti-CD36 MAbs or IRBC resulted in stimulation of the respiratory burst as measured by reduction of nitroblue tetrazolium and generation of chemiluminescence. These effects required appropriate intracellular transmembrane signaling and were inhibited by calcium antagonists or by specific inhibitors of protein kinase C or guanine nucleotide binding regulatory proteins. Anti-CD36 MAbs also stimulated human platelets to aggregate and secrete ATP. Using a cytochemical electron microscopic technique, the presence of reactive oxygen intermediates was identified at the interface between human monocytes and IRBC. These data provide support for the hypothesis that many of the pathological changes in *falciparum* malaria result from release of reactive oxygen intermediates by monocytes when IRBC ligands interact with cell surface receptors.

- 165      PLASMODIUM SPOOROZOITE INTERACTIONS WITH MACROPHAGES IN  
VITRO: A VIDEOMICROSCOPIC ANALYSIS. J.P. Vanderberg\*, P.  
Goldie, and M.J. Stewart. Dept. of Medical and Molecular  
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There is considerable controversy over the role of macrophages in clearance of sporozoites from the blood and in possibly enhancing sporozoite invasion of the liver: (1) Are sporozoites phagocytosed by macrophages or do they actively invade? (2) Do liver macrophages transport sporozoites from the blood to the hepatocyte? (3) Can internalized sporozoites evade the microbicidal mechanisms of macrophages? We used videomicroscopy to investigate *in vitro* interactions between *Plasmodium berghei* sporozoites and macrophages (resting vs. gamma interferon-activated) from rats and humans. Our results showed that sporozoites are clearly capable of direct active invasion of macrophages as well as being phagocytosed by macrophages. Active sporozoite invasion takes about ten seconds and involves gliding motility and energetic thrashing movements by the entering sporozoite. Phagocytosis takes several minutes during which time the sporozoite becomes enveloped by macrophage filipodia. The relevance of these two types of entry will be discussed in context with the requirement that successful sporozoites be able to evade destruction by macrophages during liver invasion.

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MOVEMENT OF A FALCIPARUM MALARIAL PROTEIN THROUGH THE ERYTHROCYTE CYTOPLASM TO THE ERYTHROCYTE MEMBRANE IS ASSOCIATED WITH LYSIS OF THE ERYTHROCYTE AND RELEASE OF GAMETES  
Isabella A. Quakyl\*, Yoshitsugu Matsumoto, Richard Carter, Rachanee Udomsangpetch, Masamichi Aikawa, and Louis H. Miller. \*Malaria Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; Case Western Reserve University, Institute of Pathology, 2085 Adelbert Road, Cleveland, OH 44106; Department of Genetics, University of Edinburgh, West Mains Road, Edinburgh, EH 9 3JN, Scotland; Department of Immunology, University of Stockholm S-106, 91 Stockholm, Sweden.

**Abstract:** Erythrocytes containing mature gametocytes of Plasmodium falciparum circulate in the blood until they are ingested by a mosquito, an event that triggers gametogenesis and lysis of the infected erythrocyte. It was previously shown that a parasite protein (Pf155/RESA) accumulated in the erythrocyte cytoplasm next to the parasitophorous vacuolar membrane (Masuda et al., Mol. Biochem. Parasitol., 19:213-222, 1986). Using a monoclonal antibody to Pf155/RESA and rabbit sera to two different repeat peptides of Pf155/RESA, immunoelectronmicroscopy and we have studied the location of Pf155/RESA after induction of gametogenesis by immunofluorescence. Five minutes after triggering gametogenesis, the parasitophorous membrane no longer surrounds the parasite, bringing the parasite membrane in contact with the erythrocyte cytoplasm. Clear spaces appear throughout the hemoglobin-rich host cytoplasm; Pf155/RESA is now localized in the cytoplasm directly surrounding the spaces. No membrane exists between the spaces and the erythrocyte cytoplasm. The spaces with surrounding Pf155/RESA protein extend to the erythrocyte membrane. After lysis of the erythrocyte membrane (15 min after triggering gametogenesis), the protein is distributed along the erythrocyte membrane and throughout the space between the gamete and the erythrocyte membrane. The mechanism by which the Pf155/RESA remains aggregated around the spaces and its role in erythrocyte lysis are unknown. It is of interest that the parasite uses the same molecule during invasion of erythrocytes and during release of gametes from infected erythrocytes.

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### NEW WORLD PRIMATES IN DEVELOPMENT AND EVALUATION OF MALARIA VACCINES.

W.E. Collins,\* T.K. Ruebush II and C.C. Campbell. Malaria Branch, CDC, Atlanta, GA.

Non-human primates have been expected to play an important role in the vaccine development process, both with regard to pre-human vaccine trials and in defining the immune processes associated with protection. Of the various species tested, Aotus and Saimiri monkeys were shown to support best the development of Plasmodium falciparum, P. vivax and P. malariae. Studies were conducted at CDC to determine susceptibility, prepatent periods, and maximum parasitemias using various types of monkeys and strains of parasites. The best primate-parasite combinations presently available for assessing asexual blood-stage P. falciparum vaccines are Bolivian Saimiri or A. lemurinus griseimembra from Colombia using several different parasite strains. Aotus nancymai are usable with restricted strains of parasites. The only primate-parasite combination for assessing sporozoite-induced immunity to P. falciparum is A. lemurinus griseimembra using the Santa Lucia strain. The most suitable combination for testing sporozoite-induced immunity P. vivax is the S. boliviense monkey using the Sal I strain; it is also the best model for blood-stage vaccine studies with P. vivax. No satisfactory models for testing vaccines directed against P. malariae have been defined. By committing resources to the development of a few standardized primate models, the testing of vaccines and studies related to vaccine development can be carried out in the most cost-effective manner. Supported by AID PASA  
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- 168 ASSESSMENT OF NATURALLY ACQUIRED RESISTANCE TO BLOOD STAGE P. FALCIPARUM IN IRIAN JAYA, INDONESIA. T.R. Jones,\* J.K. Baird, H. Basri, Purnomo, S. Masbar and F.P. Paleologo. U.S. Naval Medical Research Unit No. 2 Detachment, Jakarta, Indonesia.

Malaria prevalence and surveillance studies were performed in the village of Arso PIR in Irian Jaya, Indonesia. The village contains a population of people who have had lifelong exposure to endemic malaria (Irianese natives) and another population who arrived from a nearly malaria-free area 18 months before the study began (Javanese transmigrants). At the outset of this study, about 50% (473) of the villagers were screened by blood films for malaria. Prevalence rates for all malaras were significantly higher in transmigrant adults (>15 years) than in the Irianese natives (51 vs. 37%). Children had higher rates than adults in both populations. Similar patterns emerged when P. falciparum (P.f.) and P. vivax prevalence rates were analyzed separately. Spleen sizes correlated directly with prevalence rates in each age group. Twenty seven percent of the transmigrants parasitemic with P.f. carried gametocytes while only 10% of parasitemic Irianese natives were gametocyte carriers ( $p=0.013$ ). In contrast, when 240 people (2 to 60 years) were subsequently screened biweekly for 4 months, transmigrants of all age groups had parasitemias more frequently than Irianese subjects in corresponding age groups. Transmigrants again demonstrated a higher rate and density of P.f. gametocytemia. Transmigrants were ill more often when parasitemic with P.f. and their symptoms more severe. These results demonstrate greater resistance to parasitemia and illness in Irianese natives compared to transmigrants when both groups are exposed to an equal hyper or holoendemic malaria infection pressure.

- 169 USE OF DNA PROBES AS EPIDEMIOLOGIC TOOLS IN MALARIA RESEARCH  
R.H. Barker jr\*, T. Banchongaksorn, and D.F. Wirth. Harvard School of Public Health, Boston, MA. and the Malaria Division, Ministry of Public Health, Bangkok, Thailand.

We have previously reported on the characterization of DNA probes specific for P. falciparum, and upon development of a simple method for their application to lysed blood samples. In the present study we examine use of the DNA probe method for epidemiological studies involving large numbers of patients. More than 10,000 blood samples from villagers in Northern Thailand were examined in two separate studies. Results from DNA hybridization were compared with those from conventional microscopic examination. Both sensitivity and specificity of the DNA probe method compare favorably with those of microscopy, while the DNA probe method offers significant advantages for processing large numbers of samples. Results of microscopy and DNA hybridization differed in less than 0.6% of cases studied.

H: MALARIA - EPIDEMIOLOGY

170 ANTIBODY RESPONSE TO SPOROZOITES IN NATURALLY ACQUIRED  
PLASMODIUM VIVAX MALARIA IN THAILAND.

H.K. Webster, C. Wongsrichanalai, J.B. Gingrich, B. Permpnich, A. Suvarnamani, S. Tulyayon, W.R. Ballou and R.A. Wirtz. U.S. Army Medical Component, AFRIMS, Bangkok, Thailand and Walter Reed Army Institute of Research, Washington, D.C., USA.

Antibodies to sporozoites of P. vivax (PV) and the role of these antibodies in protection against malaria have not been systematically studied. An understanding of anti-sporozoite antibodies in natural infection is important to the design of a malaria vaccine. We conducted a longitudinal study of 200 Thai villagers in a malaria endemic area of southeastern Thailand. Serum samples and malaria smears were collected monthly during Jan to Oct, 1987. Antibodies against the circumsporozoite (CS) protein were measured using an ELISA with NS1<sub>1</sub>V20 as the capture antigen. Monthly PV prevalence varied from 5 to 22% and the inoculation rate was 0 to 0.04 infective bites/day during the study period. CS antibody was detected in 46-71% of the population. The percentage of CS antibody responders increased with age. A risk analysis was done to assess CS antibody protection. The age adjusted risk ratios (responders vs non-responders) were 0.71 (Aug), 0.71 (Sep), and 1.11 (Oct), all with 95% CIs that included 1, suggesting that responders were as likely as non-responders to develop PV malaria. Levels of CS antibody occurring in natural infections do not appear protective.

171 ONGOING SERO-EPIDEMIOLOGICAL STUDIES ON HUMAN PLASMODIA IN THE STATE OF PARA, AMAZON BASIN, BRASIL. M.E. Arruda\*, A.H. Cochrane, E.H. Nardin, and R.S. Nussenzweig. Department of Entomology, Oswaldo Cruz Foundation, Rio de Janeiro, Brasil, and Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY.

A sero-epidemiological survey was carried out during 1986-1987, the aim of which was to determine the prevalence of P. falciparum, P. vivax, and P. malariae/P. brasilianum in different Indian tribes located along the Xingu river. Levels of parasitemias detectable by Giemsa-stained thin blood films were very low. Sera were collected by venous puncture for determination of both anti-blood stage and anti-sporozoite antibodies against the various malarial species. Two different immunological techniques were used. An indirect immunofluorescent assay (IFA) used either air-dried blood stage parasites or glutaraldehyde-fixed sporozoites as antigen. For the ELISA we used synthetic peptides corresponding to the respective circumsporozoite (CS) repeats. Data obtained from two tribes, the Arara and the Assurini, are presented. We found that a high percentage of adults of both tribes were positive for antibodies against both blood stages and sporozoites of all three plasmodial species. Anti-blood stage titers were highest against P. falciparum. The most striking finding was that almost 90% of the Assurini had antibodies against P. malariae/P. brasilianum sporozoites as determined by both the IFA and the ELISA. In contrast, approximately 45% of the Arara had anti-sporozoite antibodies against P. malariae/P. brasilianum. The possibility of a monkey population serving as a reservoir for P. malariae/P. brasilianum is discussed.

H: MALARIA - EPIDEMIOLOGY

- 172 NATURALLY ACQUIRED ANTIBODIES TO RING-INFECTED ERYTHROCYTE SURFACE ANTIGEN (RESA) AND CIRCUMSPOROZOITE PROTEIN OF PLASMODIUM FALCIPARUM: LACK OF ASSOCIATION WITH PROTECTION AGAINST NEW MALARIA INFECTIONS IN CHILDREN IN WESTERN KENYA. A.D. Brandling-Bennett,\* G.H. Campbell, P. Nguyen-Dinh, P.M. Procell, J.A. Rubatsky, J.S. Odera, C.O. Osanga, and J.B.O. Were. Kenya Medical Research Institute and Division of Vector Borne Diseases, Nairobi, Kenya; and Malaria Branch, Division of Parasitic Diseases, CDC, Atlanta, GA.

From May to August 1987, we followed 238 children aged 3 to 8 years in western Kenya for the development of new Plasmodium falciparum infections after treating them with Fansidar<sup>R</sup>. During 15 weeks, 91% had at least one new infection, and 14% had 2 or more infections. Attack rates among susceptible children averaged 31% per week during July. The proportions of children with antibodies to the synthetic peptides of RESA, (EENV)<sub>5</sub>, (EENVEHDA)<sub>4</sub>, (DDEHVEEPTVA)<sub>3</sub>, and the circumsporozoite protein, (PNAN)<sub>5</sub>, as detected by ELISA, were 25%, 51%, 51%, and 27%, respectively. There was no difference in attack rates between children with and without antibodies to any of these peptides. There was no association between antibody, as measured by mean absorbance values, and incidence of parasitemia, presence of fever, other symptoms, or level of parasitemia. Naturally acquired antibodies to P. falciparum antigens expressed in the red cell membrane (RESA) or to the circumsporozoite antigen PNAN, which are vaccine-candidates, do not appear to confer protection from new infections to children in western Kenya. Supported by USAID PASA BST-0453-P-HC-2086-03.

- 173 ANTIBODIES TO THE PLASMODIUM FALCIPARUM RING-INFECTED ERYTHROCYTE SURFACE ANTIGEN (RESA): SEASONAL PREVALENCE IN TWO KENYAN VILLAGES. P. Deloron,\* G.H. Campbell, A.D. Brandling-Bennett, J.M. Roberts, I.K. Schwartz, S.J. Odera, and C.O. Osanga. Division of Parasitic Diseases, CDC, Atlanta, GA; INSERM U-13, Paris, France; Kenya Medical Research Institute and Division of Vector-Borne Diseases, Kenya.

To investigate the role of antibodies to RESA under conditions of natural exposure to P. falciparum, we conducted two cross-sectional surveys involving 954 individuals living in two villages of western Kenya, Asembo Bay (AB) and Got Nyabondo (GN). In both villages malaria is hyperendemic with an anopheline density 7 times higher in AB than in GN. Blood samples were collected for parasitologic examination (469 individuals were infected with P. falciparum). Serologic testing was performed using a FAST-ELISA technique, with 3 synthetic peptides representing the repeat sequences of RESA: a) (EENV)<sub>5</sub>, b) (EENVEHDA)<sub>4</sub>, and c) (DDEHVEEPTVA)<sub>2</sub>. At the end of the rainy season, 45%, 73%, and 72% of the individuals in AB had antibodies reacting with peptides A, B, and C respectively. In GN, the rates were 44%, 67%, and 56%. Positivity rates increased with age. Presence of P. falciparum parasitemia in individuals was inversely related to antibody response to peptide B, but not peptides A or C. Before the next rains, the positivity rates and the levels of reactivity were unchanged in AB, while they had decreased in GN (except with peptide C). Seasonal variation in antibody levels and positivity rates were, therefore, only apparent in GN, the village with lower malaria transmission. Supported in part by USAID PASA BST-0453-P-HC-2086-03.

## H: MALARIA - EPIDEMIOLOGY

- 174 ANTIBODY TO RESA IN NATIVES AND TRANSMIGRANTS LIVING IN ARSO PIR, IRIAN JAYA. J.K. Baird,\* T.R. Jones, B. Leksana, and B.A. Annis. U.S. Naval Medical Research Unit #2, Jakarta Detachment, APO San Francisco 96356-5000

An ELISA for Plasmodium falciparum ring-infected erythrocyte antigen (RESA) was used to obtain optical density values from sera of 120 life-long residents of Irian Jaya and from 120 age matched transmigrants from Java who arrived 18 months prior to evaluation. Volunteers were subsequently followed for development of parasitemias twice a month for four months. Even though the Javanese transmigrants proved to be more susceptible to blood stages of P. falciparum, they had consistently higher RESA optical density values in all age groups. Independently of ethnic group, antibody to RESA appeared dependent upon age and frequency of exposure: 2-5 years olds who did not become infected showed optical densities near baseline (0.120), whereas 2-5 years olds who became positive at least twice had much higher optical densities (0.390). Conversely, adults who did not develop parasitemias had higher optical densities (0.590) than those who were positive at least twice (0.460). Among Javanese, there was a negative correlation between RESA optical density and both number of positive smears ( $r=-0.9984$ ) and number of episodes of malaria symptoms ( $r=-0.9665$ ). The results suggest that levels of antibody to RESA do not correlate with protection unless history of exposure is considered.

- 175 DETECTION OF ANTIBODIES IN HUMANS OF DIFFERENT MALARIA-ENDEMIC REGIONS TO SYNTHETIC PEPTIDES DERIVED FROM PLASMODIUM FALCIPARUM EXOANTIGENS. S. Montenegro-James,\* M.A. James, S.-J. Ma, I. Kakoma, O. Noya, F. Riggione, and M. Ristic. Dept. of Veterinary Pathbiology, Univ. Illinois, Urbana, IL, and Universidad Central de Venezuela, Instituto de Medicina Tropical, Caracas, Venezuela.

Four synthetic peptides (29mer=C3, C2, C5 and C10) were constructed from internal chymotryptic digests of a 70 kd P. falciparum Indochina I exoantigen. The antigenicity of the peptides was studied by enzyme-linked immunosorbent assays, in which the peptides were either coupled to bovine serum albumin and used as coating antigens, or applied alone (unconjugated) onto nitrocellulose discs for DOT-ELISA analysis. A serum sample was considered positive for a peptide when the absorbance value was greater than the mean optical density plus 2 standard deviations obtained with sera from healthy individuals (controls). Sera from individuals living in endemic malarious regions of Venezuela, Africa and S.E. Asia; from individuals infected with P. vivax and other parasites; sera from volunteers experimentally infected with P. falciparum and sera from healthy U.S. volunteers were analyzed. Of 118 serum samples from individuals with known history of malaria, reactivity was as follows: Venezuela (29mer=73%, C2=60.3%, C10=30.8%, C5=23.1%), Africa (29mer, C2, C5=90%, C10=70%), S.E. Asia (C2=100%, 29mer=86.7%, C10=66.7%, C5=60%). The specificity of the peptide-ELISA was demonstrated by the lack of reactivity with antisera to other parasites. Cross-reactivity was found only with P. vivax. Responses to the 29mer and C2 peptides appeared at an earlier age as indicated by 100% reactivity in Venezuelan children between 0-4 years, and 71% at the 5-9 yr. age range for both peptides.

176 LIPID STIMULATION OF SCHISTOSOMA MANSONI  
CERCARIAE IS MEDIATED BY ARGENTOPHILIC PAPILLAE ON  
CERCARIAL SURFACES.

CL King\* and GI Higashi. Laboratory of Parasitic Diseases, NIH, Bethesda, MD and The Department of Epidemiology, The University of Michigan, Ann Arbor, MI.

Certain long chain polyunsaturated fatty acids (FA) found on mammalian skin trigger cercariae to penetrate and transform into schistosomules; however the mechanism by which FAs stimulate cercariae is unknown. In order to determine whether argentophilic papillae concentrated at the apical region of the cercariae are the chemoreceptors that mediate cercarial response to FAs, a system was used to assess the proportion of cercariae that penetrated a 0.25% agar matrix in the presence (61%) and absence (2.3%) of linolenic acid at 62.5 ug/ml. Silver nitrate which selectively binds to cercarial papillae (Short and Cartrett, J. Parasitol. 59: 1041, 1973) is nontoxic (at 15.6 ug/ml used in this study) as demonstrated by the ability of Ag nitrate treated cercariae to mature successfully into adult worms (8.8% maturation compared to 10.2% of untreated controls, n=5) after subcutaneous injection. When Ag nitrate was added to cercarial suspensions, penetration into linolenic impregnated agar was significantly inhibited (80.8%). This inhibition was reversed by washing cercariae free of Ag nitrate.

These data, as well as the observation that both argentophilic papillae and cercarial response to FAs disappeared within 3 to 4 hours after mechanical conversion to schistosomules, implicate argentophilic papillae on cercariae as chemoreceptors for lipid stimulation.

177 THE EFFICACY OF TOPICALLY APPLIED NICLOSAMIDE AT PREVENTING  
SCHISTOSOMIASIS MANSONI IN RHESUS MONKEYS.

\*R. E. Miller, L. Lightner, C. B. Clifford, W. A. Reid, Jr., D. Jones, and K. R. Witter. Walter Reed Army Institute of Research, Washington, DC.

We previously reported the 100% anti-penetration efficacy of a single topical application of niclosamide (2',5-dichloro-4'-nitro-salicylanilide) 24 hr before exposure of mice to Schistosoma mansoni cercariae (SmC). In the current study, the arms of 3 groups of Rhesus monkeys (Macaca mulatta) were treated with a single wipe application of niclosamide (1% W/V in absolute ethanol) or ethanol alone 1,3 or 7 days pre-exposure to SmC. Twenty min after treatment, all of the arms of the monkeys in the treatment groups were washed with continuously flowing tepid tap water for 1 hr. One monkey was used as the infection, untreated control. All monkeys were infected at the same time by exposing their forearms to  $527 \pm 11$  (SD) SmC in 3L of dechlorinated water for 1 hr. Seven weeks after exposure to SmC, the monkeys were perfused for the recovery of adult worms. The treated arms and vital organs of the monkeys were also examined for evidence of any infection or pathology resulting from the niclosamide treatment. The average worm burden for the pooled treatment and infection control monkeys was 55 adult parasites. Only one niclosamide-treated monkey was infected. This monkey was from the 3 day pre-treatment group. It had one male and one female worm. There was no pathology associated with the compound in the skin or in any of the vital organs. The 99% efficacy of niclosamide at preventing schistosomiasis mansoni for 7 days in a primate model demonstrates the potential human use of niclosamide as a simple-to-use topical prophylactic against schistosomiasis mansoni.

I: SCHISTOSOMIASIS - GENERAL

178 RISING SALINE CONCENTRATION SIGNALS CERCARIAE of *S. MANSONI*  
TO TRANSFORM TO SCHISTOMULA.

John C. Samuelson\* and Lincoln Stein, Departments of Tropical Public Health, Harvard School of Public Health, and of Pathology, Brigham and Women's Hospital, and Committee on Cell and Developmental Biology, Harvard Medical School, Boston, MA.

Cercariae (Cerc) of *S. mansoni* are shed from the snail, swim through pond water (PW), penetrate the skin of the mammalian host, and rapidly remodel their surface and transform into schistosomula (Schist). Previously, we showed that Cerc transform to Schist *in vitro* when Cerc are incubated in 300 mOsm saline (the mammalian osmolarity), and that transformation is inhibited by 1 mM eserine sulfate (ES), an acetylcholinesterase inhibitor (Samuelson and Caulfield, 1985, *J. Cell Biol.* 100:1423). Here, we have further defined the stimuli for transformation by incubating Cerc teased from the snail hepatopancreas in defined concentrations of saline. Parasites were then labeled with fluorescent Concanavalin A, a light microscopic marker of transformation to Schist. A rapid increase in salinity from 10 to 120 mOsm (the snail osmolarity) or from 120 to 300 mOsm triggered transformation, while organisms constantly exposed to 120 mOsm did not transform. These results show that 1) exposure to PW is not required for transformation and 2) the magnitude of change of saline rather than the absolute saline concentration is important. Transformation was inhibited when Cerc were incubated with ES during a 10 min time when the osmolarity was raised. 2-dimensional O'Farrell gels of <sup>35</sup>S-methionine labeled parasites showed that protein synthesis by Cerc inhibited from transforming by ES was more similar to that of untreated Cerc than to that of Schist. The latter results suggest that ES inhibits surface changes as well as changes in protein synthesis when the Cerc transform to Schist.

179 EXPRESSION AND ROLE OF CYSTEINE PROTEINASES IN *IN VITRO* CULTURED  
*SCHISTOSOMA MANSONI* SCHISTOSOMULA. Cynthia L. Chappell, Marc H. Dresden and Katherine S. Zerda. Department of Biochemistry, Baylor College of Medicine, Houston, TX.

The adult stage of *Schistosoma mansoni* utilizes host hemoglobin as a nutrient source. A proteolytic enzyme (SMw32) that has "hemoglobinase" activity is secreted into the parasite gut where it is rapidly activated by glutathione released from host red blood cells. In this report the expression of the SMw32 proteinase in developing schistosomula has been correlated with digestive tract development. A dramatic rise in enzyme activity is observed as early as Day 8-10 of culture. Indirect immunofluorescence with an anti-SMw32 monoclonal antibody showed that the proteinase is found throughout the developing cecum. No evidence of the SMw32 proteinase was found in eggs, cercariae or in newly transformed schistosomula. The importance of cysteine proteinases to parasite development was also studied using a specific, irreversible inhibitor, Ep-459. In cultures where cysteine proteinase activity was completely inhibited by Ep-459, 75% of the schistosomula failed to survive the 18-day study period. Moreover, the survivors had significantly less growth (body length) than normal controls. These data suggest that the SMw32 proteinase is a developmentally regulated enzyme and that cysteine proteinase activity is essential in providing nutrients for the growth and survival of this parasite in its mammalian host. Thus, this proteinase may be an important target for chemotherapeutic intervention. Supported by NIH grant AI 15864.

I: SCHISTOSOMIASIS - GENERAL

180 ISOLATION AND CHARACTERIZATION OF A CYSTEINE PROTEINASE FROM FASCIOLA HEPATICA ADULT WORMS.

A. A. Rege\*, P. R. Herrera, M. Lopez, and M. H. Dresden. Dept. of Biochemistry, Baylor College of Medicine, Houston, TX, USA; Dept. of Biochemistry and Institute of Tropical Medicine, Universidad Peruana Cayetano Heredia, and Dept. of Biochemistry, Universidad de San Marcos, Lima, Peru.

Adult Fasciola hepatica worms contain proteinases capable of degrading hemoglobin, immunoglobulins, and collagen. Here we report isolation and biochemical characterization of a cysteine proteinase from acidic extracts of these worms. The enzyme was purified to homogeneity by cation exchange and molecular sieve high performance liquid chromatography. The apparent molecular weight of the native (molecular sieve HPLC) and the denatured (SDS-polyacrylamide gel electrophoresis) enzyme was 14,500 daltons. Activity was assessed by employing synthetic peptide substrates, such as carbobenzoxy-phenylalanyl-arginyl-7-amino-4-trifluoromethyl coumarin, commonly used to assay other cysteine proteinases. The proteinase was maximally active at pH 6.0 with >50% of the activity detected between pH 4.5 and 7.6. The purified enzyme was stable in 1 M urea and in acidic pH at 25°C for several hours, at 4°C for several days, and at -20°C for several months. Inhibition of activity at pH 5.5 was seen only with compounds known to inhibit cysteine proteinases. No effect was noted with inhibitors of aspartic, serine, or metalloproteinases. Supported by the U.S. Agency for International Development.

181 PROTEIN PHOSPHORYLATION DURING TRANSFORMATION OF CERCARIAE TO SCHISTOSOMULA OF SCHISTOSOMA MANSONI.

P.M. Wiest,\* W.D. Bowen, and G.R. Olds. Brown University and The Miriam Hospital, Providence, Rhode Island.

A critical period in the life cycle of Schistosoma mansoni is the transformation of free-living cercariae to schistosomula since this is the time when the parasite must rapidly adapt to a parasitic existence within the human host. In vitro, both an elevation in temperature to 37°C and salt-containing medium stimulate transformation. The biochemical events that regulate this transition, however, are unknown. The possible involvement of protein phosphorylation was investigated by incubating cercariae with 200 uCi of <sup>32</sup>P-phosphate in phosphate-free medium for 3 hr at 37°C and analyzing solubilized parasites by SDS-polyacrylamide gel electrophoresis and autoradiography. A labelled 14 kDa protein was identified in newly-transformed schistosomula but not cercariae. The rate of phosphorylation was temperature dependent since the 14 kDa band was phosphorylated within 1 hr when parasites were incubated in isotonic culture medium at 37°C but not at 23°C. Phosphorylation did not occur when parasites were incubated in water at 37°C. Sensitivity of the 14 kDa band to alkaline hydrolysis (1 N NaOH, 55°C, 2 hr) suggests phosphorylation of serine. The cAMP analogue 8-bromo-cAMP did not stimulate phosphorylation in cercariae. Furthermore, no differences in cAMP levels, as determined by radioimmunoassay, were found during transformation. These data suggest that a cAMP-independent phosphorylation of a 14 kDa protein is associated with transformation of cercariae to schistosomula.

I: SCHISTOSOMIASIS - GENERAL

182

ULTRASONOGRAPHICAL EVALUATION OF THE MORBIDITY INDUCED BY SCHISTOSOMA MANSONI INFECTION IN CHILDHOOD

R. Kardorff, E. Doehring-Schwerdtfeger, I. M. Abdel-Rahim, G. Mohamed-Ali, Ch. Kaiser, M. El Sheikh, and J.H.H. Ehrich, Kinderklinik Med. Hochschule, 3 Hannover 61, FRG and University of Gezira, Wad Medani, Sudan

Sonographical and sonomorphometrical assessment of liver and spleen sizes in 534 Sudanese schoolchildren with *Schistosoma mansoni* infection were compared to age-matched controls ( $n = 60$ ). Sonomorphological examinations allowed differentiation of three degrees of periportal fibrosis (PF). 55 patients had PF grade I (mean egg output  $53 \pm 27$  SD per g stool). 124 had grade II ( $241 \pm 111$  SD) and 25 patients grade III ( $1060 \pm 600$  SD). The liver sizes in sternal-, midclavicular and anterior axillary line, splenic volume and length were significantly larger in patients with PF than in controls ( $n < 0.005$ ) and study patients without PF ( $n < 0.01$ ). However, there were no significant differences of liver and spleen sizes between PF grades I, II and III. 420 study patients and 47 controls could be sonographically reexamined 6 months after therapy with praziquantel. The percentage of grade III PF decreased from 4.6 % to 1.7 %; grade II from 22.4 % to 6.6 %. There was a shift from high degrees of PF to grade I resulting in an increase from 9.7 % to 29.4 %. 15 % of controls had PF grade I. We conclude: 1. Morbidity induced by *S. mansoni* infection was high in Sudanese schoolchildren who showed a substantial amount of sonographically detectable periportal fibrosis. 2. Partial reversibility of liver lesions was observed half a year after therapy with praziquantel

(Supported by Parasitic Disease Programme, WHO and Edna McConnell Clark Foundation, New York).

ULTRASONOGRAPHY OF SCHOOL CHILDREN WITH SCHISTOSOMIASIS MANSONI

183 M.F. Abdel-Wahab,\* G. Esmat, S.I. Narooz, A. Yosery, J.P. Struewing, and G.T. Strickland. Cairo University Faculty of Medicine, Cairo, Egypt and University of Maryland School of Medicine, Baltimore, MD.

Abdominal ultrasonography (US) can differentiate between schistosomal fibrosis and cirrhosis. It is also a noninvasive test and can be performed in the field. This study's goal was to determine if US could detect early changes of schistosomal fibrosis and provide information on morbidity in 309 school children living in a village endemic for *S. mansoni*. They had an average age of 14.6 years and a prevalence and intensity of ova in their stools as follows: none (42%), light (26%), moderate (21%), heavy (11%), infections. Hepatomegaly (53%) and palpable spleens (35%) were common physical findings. A history of exposure to water correlated with both the prevalence and intensity of infection. Few symptoms or signs were associated with density of infection; blood in the stools and liver span in the MCL being the most significant. US demonstrated frequent hepatomegaly (80%) and splenomegaly (49%); liver span and spleen size correlated with density of infection. Thickening of portal tracts and portal vein walls were the most reliable US correlates of density of infection. US is a more valuable determinate of *S. mansoni* infection and morbidity in communities than the classical parasitological and clinical measurements. This is particularly important since the widespread use of antischistosomal drugs and increasing prevalence of disease caused by viral hepatitis make these standard parameters of schistosomal endemicity less reliable than before.

J: WORKSHOP: LATE BREAKING ADVANCES IN MOLECULAR BIOLOGY

184-190: To be available at the Registration Desk Tuesday morning, December 6.

K: ARBOVIROLOGY - DIAGNOSIS AND TAXONOMY

- 191 EVALUATION OF FILTER HYBRIDIZATION FOR THE DETECTION OF RIFT VALLEY FEVER VIRUS RNA IN HUMAN SERUM FROM THE RECENT MAURITANIA EPIDEMIC.  
F.K. Knauert,\* J.M. Meegan, A. Jouan, B. Le Guenzo, T.G. Ksiazek, C.J. Peters, and J.P. Digoutte. Disease Assessment Division, USAMRIID, Fort Detrick, Frederick, MD 21701 and Pasteur Institute, Dakar, Senegal.

Human serum collected during the recent Rift Valley fever virus (RVFV) epidemic that occurred in southern Mauritania during the 1987 rainy season were tested by a filter hybridization to evaluate this procedure's diagnostic potential. Samples were treated with polyethylene glycol to precipitate virus and immune complexes. This treatment increases sensitivity by coincidentally reducing the amount of interfering, nonspecific, macromolecular contamination in the sample. The precipitate was collected by centrifugation, and resolubilized in a proteinase K solution to further reduce protein contamination. After the proteinase K treatment, samples were made 6X saline-sodium citrate buffer (SSC)/7.5% formaldehyde, incubated at 60°C and filtered through nitrocellulose paper. Previous work with model systems indicated that this was the optimal way to prepare serum samples for hybridization. The filtered samples were then subjected to standard hybridization conditions using a nick-translated plasmid containing RVFV M-segment RNA sequences as the probe. Using virus isolation as the criterion to judge sensitivity and specificity, we found the hybridization procedure to be 15% sensitive. By comparison, an antigen-detection ELISA run in parallel was found to be 30% sensitive. Both these procedures were 98-100% specific. There was a correlation between our ability to detect virus by either of these procedures with the absence of RVFV-specific antibody in the sample. Encouraged by the ability of this procedure to detect viral RNA in field specimens, we are testing alternate assay formats to improve the procedure's sensitivity.

- 192 PRELIMINARY EVIDENCE THAT PHLEBOTOMUS FEVER AND UUKUNIEMI SEROGROUP VIRUSES ARE ANTIGENICALLY RELATED: PROPOSED INCLUSION IN AN EXPANDED GENUS PHLEBOVIRUS.

Charles H. Calisher,\* David H. L. Bishop, and R. E. Pettersson. Centers for Disease Control, Fort Collins, Colorado, NERC Institute of Virology, Oxford, England, and Ludwig Institute of Cancer Research, Karolinska Institute, Stockholm, Sweden.

Five genera presently are recognized within the family Bunyaviridae. Two of these, Phlebovirus and Uukuvirus, are comprised of single serogroups of viruses, the Phlebotomus fever (PHL) serogroup and the Uukuniemi (UUK) serogroup, respectively. PHL serogroup viruses have been isolated mainly from sandflies and mosquitoes, whereas UUK serogroup viruses have been isolated mainly from ticks. Published and unpublished studies of the mode of replication, number and size of S RNA gene products, conservation of amino acid sequence homology of the M RNA products and of cysteine positions in the glycoproteins, and virus ultrastructure suggested that viruses of these serogroups might also be antigenically related, although no such relationship had been found. We found minimal inhibition of hemagglutination of PHL viruses by antibody to UUK viruses. Indirect immunofluorescence provided more evidence that viruses of these serogroups are related antigenically. We will present results of neutralization tests and enzyme-linked immunosorbent assays supporting the placement of the uukuviruses in the genus Phlebovirus.

K: ARBOVIROLOGY - DIAGNOSIS AND TAXONOMY

193 NUCLEIC ACID BLOT HYBRIDIZATION TECHNIQUES FOR DETECTION AND SURVEILLANCE OF BLUETONGUE VIRUS

R.J. Schoepp,\* J.F. Bray, F.R. Holbrook, C.D. Blair, P. Roy, and B.J. Beaty. Department of Microbiology, Colorado State University, Fort Collins, CO; USDA-ARS, Laramie, WY; and Department of Environmental Health, University of Alabama at Birmingham, Birmingham, AL.

Surveillance and diagnosis of bluetongue virus (BTV) in vectors and vertebrate hosts is a difficult, laborious, time consuming, and expensive procedure. Virus isolation in cell culture and embryonated chicken eggs currently are the methods of choice. Nucleic acid (NA) blot hybridization was investigated as a possible alternative. NA hybridization is sensitive, specific and useful in situations where virus isolation or serodiagnosis is difficult.

Two blot hybridization techniques were compared in their abilities to detect BTV nucleic acids. Samples were extracted, bound to a solid support, nylon membrane, and were detected with a labeled NA probe. The first technique detects NA bound directly to the solid support using a labeled RNA transcription probe. The second technique, sandwich hybridization utilizes a single stranded DNA catcher sequence bound to the solid support that captures the viral nucleic. The detection is accomplished again by use of a labeled RNA transcription probe. Each technique was used to detect BTV infected cells and Culicoides variipennis pools constructed of varying numbers of BTV infected and uninfected flies.

Both NA hybridization techniques are able to detect as few as one infected fly in a pool of fifty or approximately  $2.5 \log_{10}$  TCID<sub>50</sub> per ml. The sensitivity of each method will be compared and advantages and disadvantages discussed. The use of radioisotope labeled probes will be compared with biotin labeled probes.

194 DETECTION OF DENGUE VIRUS IN MOSQUITOES AND HUMAN SERUM BY NUCLEIC ACID HYBRIDIZATION.

K. Olson,\*C. Blair, R. Padmanabhan, and B. Beaty. Dept. Microbiology, Colorado State University, Fort Collins, CO. and Dept. Biochemistry, University of Kansas Medical Center, Kansas City, KS.

The development of rapid and reliable surveillance techniques is critical for monitoring dengue virus activity. Current virus isolation and identification techniques can be slow, costly, and labor intensive. We report the use of nucleic acid hybridization for the detection of dengue-2 virus in biological specimens.

Molecular hybridization techniques were developed to detect dengue-2 viral RNA in pools of intrathoracically infected Aedes albopictus and virus spiked human serum. Samples were analyzed by both direct detection and sandwich hybridization. The direct detection of covalently bound analyte to a nylon membrane was accomplished using radiolabeled strand specific RNA probes. Sandwich hybridization annealed analyte to two contiguous cDNA sequences, with one sequence subcloned into a M-13 phage and bound to nylon and the second sequence subcloned into in vitro transcription plasmid (pGEM-1) and used to detect the analyte. cDNAs used for construction of probes originated from the NS-1 and NS-5 gene regions of dengue-2 virus.

As few as one infected mosquito in a pool of 100 uninfected mosquitoes or  $2.75 \log_{10}$  TCID<sub>50</sub> per ml of this titrated pool can readily be detected by either hybridization technique. Dengue-2 spiked human serum is currently being analyzed by these hybridization techniques. Both the sensitivity and specificities of these techniques will be addressed.

K: ARBOVIROLOGY - DIAGNOSIS AND TAXONOMY

- 195 DEVELOPMENT OF A PLAQUE-IMMUNOBLOTTING PROCEDURE FOR FLAVIVIRUSES.  
P.L. Summers,\* D.R. Dubois, W. Houston Cohen, R. McN. Scott, and  
K.H. Eckels. Walter Reed Army Institute of Research, Washington, D.C.

Immunoblotting has become a valuable tool for diagnosis and immunological characterization of viruses. We have combined immunoblotting with plaquing techniques to create a sensitive assay for the titration and identification of flaviviruses. Dengue and Japanese encephalitis viruses were plaque-titrated in LLC-MK<sub>2</sub> or mosquito C6/36 cells. Upon completion of the plaque assay, the agar was removed and nitrocellulose (NC) was applied directly on the infected cell monolayer. Viral antigen from infected cells transferred to the NC blot by diffusion. The blot was developed immunoenzymatically and an insoluble precipitate formed an immunoplaque on the NC. Cell monolayers were left unfixed or the cells could be fixed with methanol causing no loss of antigen reactivity. In both cases, very efficient replicates of the plaque assay were blotted onto the NC; viral plaques and immunoplaques on the NC were comparable in number. We have successfully used the plaque-immunoblotting assay to titrate and serotype dengue viruses, and plan to use this technique to study mixed infections of flaviviruses. The applicability of this method may extend to all viruses regardless of their ability to form plaques in cell culture.

- 196 DENGUE HEMORRHAGIC FEVER IN JAKARTA, INDONESIA. C.R. Bartz, C. Maroef, Tatang K.S., Hansa W., R. Tan, A. Sie., U.S. Naval Medical Research Unit No. 2 Detachment and Sumber Waras Hospital, Jakarta, Indonesia

The purpose of this study is to define which dengue virus types are currently circulating in Jakarta, correlate virus type with clinical severity, and determine if IgM capture ELISA can be used as a reliable predictor of subsequent clinical severity. The study began in September 1987 and will continue to August 1988. During the first 8 months of the study at Sumber Waras Hospital in West Jakarta, it was evident an epidemic of large proportions was occurring. Serum samples have been collected from over 1,000 patients showing signs of dengue hemorrhagic fever. All samples will be cultured in Toxorhynchites splendens adults and TRA-284 (T.amboinensis) cells. Through April 1988, 42 Dengue type 3, 16 Dengue type 1 and 2 Dengue type 2 isolates had been obtained from these patients, although culturing is not complete. IgM capture ELISA, dengue virus culture, or modified WHO HAI serology criteria, confirmed 69% of the patients sampled during the first 4 months of the study had dengue disease. Preliminary results indicate IgM positivity may be a predictor of subsequent thrombocytopenia, shock and severe hemorrhage. Thrombocytopenia was found to occur on day 3 or 4 of hospitalization in many patients presenting with high IgM capture ELISA results. Isolation of virus from 50% of the primary patients which were positive by IgM capture but not confirmed by HAI indicate IgM capture is a useful diagnostic tool. Twenty percent of the confirmed cases are primary. Final results of the study will be presented.

K: ARBOVIROLOGY - DIAGNOSIS AND TAXONOMY

- 197 RIFT VALLEY FEVER AMONG DOMESTIC ANIMALS IN THE RECENT WEST AFRICAN  
OUTBREAK  
T.G. Ksiazek,<sup>\*1</sup> A. Jouan,<sup>2</sup> J.M. Meegan,<sup>1</sup> B. LeGuerno,<sup>2</sup> M.L. Wilson,<sup>2</sup> C.J.  
Peters,<sup>1</sup> J.P. Digoutte,<sup>2</sup> M. Guillaud,<sup>3</sup> N.O. Merzoug,<sup>4</sup> and O.I. Touray<sup>5</sup>.  
<sup>1</sup>USAMRIID, Fort Detrick, MD, USA <sup>2</sup>Institute Pasteur, Dakar, Senegal, <sup>3</sup>IEMVT,  
Cedex, France, <sup>4</sup>CNERV, Islamic Republic of Mauritania, and <sup>5</sup>Department of Animal  
Health and Production, Abuko, The Gambia.

The 1987 Rift Valley fever (RVF) virus outbreak in the Senegal River Basin came to the attention of scientists because of severe hemorrhagic disease among the human population. However, local herdsmen reported high incidence of abortion and disease in their domestic livestock. Serum samples were obtained from domestic animal populations from the area near Rosso, Islamic Republic of Mauritania, where most human disease was reported. Among these animals, antibody prevalence was as high as 85% with approximately 80% still having RVF virus-specific IgM antibodies. In contrast, human populations in the same area had RVF antibody prevalences of up to 40% with a similar high proportion also IgM positive. Sera from local breeds of livestock in coastal areas 280 km south of the epidemic area were negative for RVF virus antibodies. Thus, the use of RVF virus-specific IgG and IgM antibodies provided evidence of recent disease activity without pre-bleeds or viral isolation. Subsequently, detection of modest levels of IgG and IgM in the Ferlo region, 130 km south of the Senegal River flood plain, established the extension of RVF virus transmission into another area of the basin.

Application of these serological methods to the Gambia, 340 km south of Rosso, demonstrated antibody prevalence consistent with lower levels of transmission, but, nonetheless, evidence of recent RVF viral activity, i.e., 24% IgG positive with 6% of the positive sera also having IgM.

L: MALARIA - EXOERYTHROCYTIC ANTIGENS

- 198 PLASMODIUM FALCIPARUM SPOROZOITES RECOGNIZE A 55-60K HUMAN HEPATOCYTE PUTATIVE RECEPTOR. J. vanPelt, S.B. Aley, M.R. Hollingdale, J.-P. Verhave, and S.H. Yap. University of Nijmegen Medical School, The Netherlands; Biomedical Research Institute, Rockville, Md.

Malaria sporozoites specifically invade hepatocytes. To determine proteins on human hepatocytes which may be involved in the invasion of P. falciparum sporozoites, plasma membranes were isolated from human livers, and the proteins were extracted with CHAPS and labelled with <sup>125</sup>I/IODOGEN. The labelled proteins were incubated with P. falciparum sporozoites and cross linked with dithio-bis-succimidyl proprionate (DTSP). The crosslinked sporozoites were washed repeatedly until no further label could be released, then incubated with DTT to reverse the covalent crosslinking. Radiolabeled proteins released by reduction were separated by SDS-PAGE and detected by autoradiography. Two bands, of 55 and 20K, were detected. No such peptides were detected in parallel experiments using rat hepatocytes (not invaded by P. falciparum sporozoites). Rabbit antisera raised against electrophoretically purified 20K protein reacted with both 20 and 50K proteins on Western blots and, in preliminary experiments, reduced human hepatocyte invasion by sporozoites. These results mirror earlier findings by us that demonstrated that invasion might be correlated with the specific, saturable interaction of the CS protein N1 region to hepatoma (HepG2-A16) cell proteins of 55 and 35K (Aley, et al, J. Exp. Med. 1987). We propose that these proteins represent putative hepatocyte receptors for P. falciparum sporozoite invasion. Supported by AID contract DPE-0453-C-00-3051-00.

- 199 MONOCLONAL ANTIBODIES (MABs) REACTING WITH CIRCUMSPOROZOITE (CS) PROTEINS OF THE PLASMODIUM CYNOMOLGI COMPLEX AND OF P. KNOWLESII INHIBIT THE IN VITRO DEVELOPMENT OF EXOERYTHROCYTIC STAGES OF P. CYNOMOLGI BASTIANELLII. P. Millet,\* K.K. Kamboj, A.H. Cochrane, W.E. Collins, and P. Nguyen-Dinh. Malaria Branch, CDC, Atlanta, GA and Department of Medical and Molecular Parasitology, New York University, NY, NY.

CS protein repeat regions of sporozoites are highly immunogenic and responsible for attachment and entry of the parasite into hepatocytes. Previous studies have demonstrated antigenic diversity among the CS proteins of different strains of the simian malaria parasite Plasmodium cynomolgi. Immunologic cross-reactivities have also been observed between P. cynomolgi bastianellii and P. knowlesi (H strain). Primary cultures of rhesus monkey hepatocytes were infected with P. cynomolgi bastianellii sporozoites in the presence of MABs directed against CS protein of 5 strains of P. cynomolgi and P. knowlesi (H strain). Inhibitory activity of these MABs on the exoerythrocytic stage development was determined by the number of schizonts developed after 6 or 7 days. For the P. cynomolgi group of MABs, strain specificity was observed since only the 2 P. cynomolgi bastianellii MABs totally inhibited P. cynomolgi bastianellii sporozoites at concentrations of 500 and 50 ug/ml. However, 1 MAB against P. knowlesi showed 80% inhibition at 50 ug/ml. Only the inhibitory MABs reacted by Western blot with the CS protein of P. cynomolgi bastianellii, thus showing correlation between the immunologic results and these functional studies. Supported by USAID PASA BST-0453-P-HC-2086-03 and NRC Fellowship to PM.

L: MALARIA - EXOERYTHROCYTIC ANTIGENS

200 INHIBITION OF IN VITRO DEVELOPMENT OF PLASMODIUM BERGHEI LIVER STAGE PARASITES BY SPLEEN CELLS FROM MICE IMMUNIZED WITH IRRADIATION ATTENUATED SPOROZOITES.

D. Isenbarger\*, G. Long, W.R. Ballou, and S.L. Hoffman. Walter Reed Army Institute of Research, Washington, D.C. and Naval Medical Research Institute, Bethesda, M.D.

Mice immunized with irradiation attenuated rodent malaria sporozoites are protected against sporozoite challenge by a T cell dependent immune mechanism. To determine if antigens expressed in infected hepatocytes could be targets of this protective cellular immune response, we developed an *in vitro* system for assessing the effect of spleen cells from irradiated sporozoite immunized mice on the development of the exoerythrocytic (EE) stages of *Plasmodium berghei*. When compared to normal spleen cells, immune spleen cells (ISCs) from H-2<sup>d</sup> mice inhibit the development of late liver schizonts in H-2<sup>d</sup> hepatocytes by greater than 90%. In this system, inhibition of EE stage development appears to require antigen recognition in the context of Class I major histocompatibility complex molecules, and not to require interferon gamma. ISCs from H-2<sup>b</sup> mice inhibit the development of EE stage parasites in H-2<sup>b</sup> hepatocytes, but not in H-2<sup>d</sup> hepatocytes, and the inhibition by H-2<sup>d</sup> ISCs in H-2<sup>d</sup> hepatocytes is not reduced by addition to the cultures of rabbit anti-mouse interferon gamma. These studies demonstrate that mice immunized with irradiated *P. berghei* sporozoites develop a cellular immune response directed against antigens expressed in infected hepatocytes.

201 PLASMODIUM BERGHEI: EXPRESSION OF CIRCUMSPOROZOITE PROTEIN DURING EXOERYTHROCYTIC DEVELOPMENT IN HEPG2-A16 HEPATOMA CELLS.

Carter T. Atkinson,\* Masamichi Aikawa, Stephen B. Aley and Michael R. Hollingdale. Case Western Reserve University, Cleveland, OH and Biomedical Research Institute, Rockville, MD.

The fate of *P. berghei* circumsporozoite (CS) protein was studied by post-embedding immunoelectron microscopy in exoerythrocytic (EE) stages grown in HepG2-A16 hepatoma cells. We used monoclonal antibody (MAb) 3D11 against the *P. berghei* CS protein to localize CS antigen in developing parasites at 3, 24, 50 and 70 hours after sporozoite invasion. CS antigen or a cross reacting epitope persisted throughout EE development as a major schizont surface protein and was incorporated into the membrane of budding EE merozoites. Erythrocytic merozoites were not labeled by MAb 3D11, indicating a clear antigenic difference from EE merozoites. Significant internal labeling within 50 hour EE schizonts was associated with membrane-bounded vesicles of various sizes and with electron-dense patches under the plasma membrane. MAb 3D11 labeled the outer membrane of the internal vesicles but not an electron-dense flocculent material within their contents. Similar unlabeled flocculent material was also present in the parasitophorous vacuole (PV) space and in vesicles in the host cell cytoplasm which were adjacent to the PV. These observations suggest that *P. berghei* CS protein may play an important role in mediating host-parasite interactions during EE development.

L: MALARIA - EXOERYTHROCYTIC ANTIGENS

202 PEPTIDE DERIVED FROM THE PLASMODIUM FALCIPARUM LIVER STAGE SPECIFIC ANTIGEN ELICITS ANTIBODIES THAT REACT WITH P. FALCIPARUM SPOROZOITES AND P. BERGHEI SPOROZOITES AND EXOERYTHROCYTIC STAGES.

M.R. Hollingdale\*, S.B. Aley, A. Appiah, M. Aikawa, and C. Atkinson. Biomedical Research Institute, Rockville, Md., Case Western University, Cleveland, OH.

Recently, an antigen apparently specific to the exoerythrocytic (EE) stage of Plasmodium falciparum was cloned, sequenced, and shown to contain at least 3½ repeats of a 17 amino acid sequence EQQSDLEQERLAKEKLQ (Guerin-Marchand et al 1987). We have synthesized this peptide and immunized rabbits with the peptide conjugated to poly-L-lysine. Immune sera had an ELISA titer specific to the 17mer in excess of 1:6,000 and was positive by indirect immunofluorescence on both P. falciparum and P. berghei sporozoites to 1:1,000. In the inhibition of sporozoite invasion (ISI) assay, the ISI<sub>50</sub> of rabbit anti-17mer sera was 1:1400 against P. falciparum sporozoites, and 1:320 against P. berghei sporozoites. In colloidal gold immuno-electron microscopy, rabbit anti-17mer sera reacted with the surface of P. falciparum and P. berghei sporozoites, and the plasma membrane of P. berghei EE parasites grown in cultured HepG2-A16 hepatoma. The potential of the 17mer as a candidate anti-malarial vaccine is being tested in mice by challenge with P. berghei sporozoites. In these experiments, both EQQSDLEQERLAKEKLQ and QERLAKEKLQEQQSDLE have been synthesized and conjugated to limpet hemocyanin. Sera from all these experiments are also being tested for reactivity with P. falciparum EE parasites, and used for screening P. falciparum and P. berghei expression libraries.

Supported by AID contract DPE-0453-C-00-3051-00

203 IMMUNIZATION WITH IRRADIATED PLASMODIUM BERGHEI SPOROZOITES DOES NOT PROTECT AGAINST CHALLENGE WITH LIVER STAGE MEROZOITES: A REDEFINITION OF STAGE SPECIFICITY OF SPOROZOITE VACCINES.

G Long, L Loomis, WR Ballou, and SL Hoffman. Naval Medical Research Institute, Bethesda, MD, and Walter Reed Army Institute of Research, Washington, DC.

Immunization of mice with irradiated Plasmodium berghei sporozoites induces complete protective immunity against sporozoite challenge. The target antigens of this immune response have not been clearly defined, but may be present on exoerythrocytic (EE) liver stages of the parasite. Sera from mice immunized with 4 doses of irradiated sporozoites were depleted of antibodies to the predominant repeats of the P. berghei CS protein (DPAPPNAN and DPPPPNPN) by affinity chromatography. After depletion these sera were not reactive with sporozoites by immunofluorescence at a dilution of 1/8, but were reactive at a dilution of 1/64 with late liver stage schizonts of P. berghei, suggesting that mice immunized with irradiated sporozoites develop antibodies to both sporozoite antigens and non-sporozoite antigens expressed in EE stage parasites. If added to cultures after sporozoites have been allowed to invade, these antibodies do not inhibit the development of EE stage parasites *in vitro*. We utilized a continuous cell line derived from the livers of Balb/c mice that allows complete development of P. berghei EE stages, to determine if such antibodies inhibited infection of erythrocytes by EE stage merozoites. Irradiated sporozoite immunized and control mice were challenged with P. berghei sporozoites or with culture supernatants containing EE stage merozoites. All immunized mice were protected against sporozoite challenge, but none were protected against merozoite challenge. These data indicate that protective immunity elicited by immunization with irradiated sporozoites does not function through an effect on merozoites released from liver stages of the parasite.

M: TROPICAL VETERINARY MEDICINE

- 204 Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). T.N. Mather, M. L. Wilson, S.I. Moore, J.M.C. Ribeiro, and A. Spielman. Department of Tropical Public Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA.

We compared the contribution of white-footed mice (*Peromyscus leucopus*), chipmunks (*Tamias striatus*) and meadow voles (*Microtus pennsylvanicus*) to infection of vector ticks with the Lyme disease spirochete, *Borrelia burgdorferi*. At one location where Lyme disease is epidemic, all 3 species of rodents were found to be infected. Prevalence of infection, however, varied from 90% for mice, 75% for chipmunks, to just 5.5% for meadow voles. Infectivity of these hosts for larval *Ixodes dammini* also varied but mice were found to be the most infective, followed by chipmunks and meadow voles. Density estimates of these 3 hosts, made in 3 coastal Massachusetts locations, revealed that mice were more abundant than the other 2 rodents in areas where ticks were abundant. In addition, mice were infested more abundantly with larval *I. dammini* than the other two host species. Integrating these results, we determined each species' "reservoir potential", a novel term which describes the relative contribution made by a host species to the horizontal infection of a vector population. Our findings demonstrate that, at least in coastal Massachusetts, *P. leucopus* is the most important small mammal reservoir for Lyme disease spirochetes.

- 205 ISOLATION OF A MAJOR ANAPLASMA MARGINALE PROTEIN FOR USE IN A DOT-ELISA FOR THE SERODIAGNOSIS OF ANAPLASMOSIS. S. Montenegro-James, S.-J. Ma, and M. Ristic. Dept. of Veterinary Pathobiology, Univ. Illinois, Urbana, IL.

There is a need for a simple, rapid, sensitive and specific serodiagnostic test for bovine anaplasmosis, one of the most important diseases of cattle in the United States and many tropical countries of the world. Existing serodiagnostic assays are not entirely satisfactory in that the antigen used is a crude mixture of the organism and erythrocytic stroma. Five *A. marginale* (Florida isolate) proteins of apparent molecular weights ranging from 100kd to 20kd were isolated from SDS-PAGE gels by electro-elution and allowed to return to their native configuration during ethanol precipitation. A major protein of approx. 38kd was selected for use in a DOT-ELISA at an optimum concentration of 5 ng per dot. Serologic reactivity with the 38kd antigen was analyzed in 200 serum samples from naturally and experimentally infected cattle from the U.S. and Venezuela. Sera from 20% of experimentally infected cattle recognized the 38kd protein 1 week post-inoculation, with 100% seroconversion 2 weeks post-infection. The potential protective capability of the 38kd antigen was demonstrated by the consistently high antibody titers observed in immune animals. No false positive reactions were detected with the 38kd/DOT-ELISA. Moreover, the test showed a 97% sensitivity in contrast to a 82% sensitivity with the IFA test. N-terminal amino acid sequencing of the purified 38kd protein is in progress for the eventual construction of synthetic peptides.

## M: TROPICAL VETERINARY MEDICINE

### AN EPIZOOTIC OF URBAN CANINE RABIES IN MEXICO

206 TR Eng \*, HE Talamante, DB Fishbein, GM Baer, DB Hall, GF Chavez, JG Dobbins, J Carrasco, FJ Muro. Div of Viral Diseases, Div of Birth Defects and Developmental Disabilities, Centers for Disease Control; Servicios Medicos de Sonora, Mexico; El Paso Field Office, Pan American Health Organization

Between August 1, 1987 and February 29, 1988, 182 cases of canine rabies were confirmed in Hermosillo, Mexico ( a city with an estimated population of 580,000 people), compared to only 2 cases in the previous 7 years. The outbreak began in the southern areas of the city and involved the entire city by November. Despite the vaccination of 32,608 dogs (59% of the estimated canine population) in 1987, canine rabies persisted, especially in the lower socioeconomic areas of the city. A random survey of 912 households revealed that 47% owned dogs and that there were approximately 8 persons per owned dog. In addition, an average of 2.5 dog bites were reported for every 1,000 residents in the past year. Of those dogs bled in one area 3 months after a mass vaccination campaign, only 57% vaccinated for the first time had rabies antibodies; 82% of all dogs  $\geq$  3 months of age had been vaccinated during the past year. In a subsequent prospective study, 87% of dogs given a modified live vaccine intramuscularly (IM) had rabies antibodies 5 weeks after vaccination, compared to only 46% or 57% of dogs given suckling mouse brain vaccine IM or subcutaneously ( $p=0.02$ ). In another mass vaccination campaign on April 17, 1988, in which a different vaccine was used, 22,196 dogs were immunized that one day. The effect of this campaign is being evaluated.

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### A UNIQUE SPOTTED FEVER GROUP RICKETTSIA FROM HUMANS IN JAPAN

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Spotted fever group (SFG) rickettsiae were isolated from the blood of patients in Japan where no SFG rickettsiosis had been reported until recently. The isolates established persistent infections of Vero cells unlike other pathogenic tick-borne SFG rickettsiae. To identify the species of Japanese strains, reciprocal cross-reactions were performed among Japanese and standard strains of SFG rickettsiae by the microimmunofluorescence (micro-IF) method using mouse antisera to the strains tested. Specific differences between pairs of strains calculated from micro-IF titers demonstrated that the Japanese strains belong to a single species but differ from any other pathogenic tick-borne rickettsia. Although the Japanese strains were closely related antigenically to R. akari, a mite-borne SFG rickettsia, a monoclonal antibody specific for R. akari does not react with the Japanese strains. Western immunoblotting demonstrated distinctive mobilities of the major protein antigens. The Japanese strains were also shown to differ from the Thai tick typhus rickettsia, an isolate from ticks. The findings provide evidence that the Japanese SFG rickettsia is a new species. Transmission electron microscopy showed typical intracytoplasmic rickettsiae surrounded by an electron-lucent halo with a cell wall composed of thin outer and thick inner leaflets.

M: TROPICAL VETERINARY MEDICINE

208 ADAPTATION OF EHRLICHIA CANIS TO GROWTH IN PRIMARY HUMAN MONOCYTE CELL CULTURES.

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University of Illinois, College of Veterinary Medicine, 2001 S. Lincoln Ave.,  
Urbana, IL 61801.

Ehrlichia canis is the causative agent of canine ehrlichiosis which, until recently, has been considered to be restricted to canidae. Previous attempts to infect other animal species or to propagate the organism in cell cultures other than those of canine origin have failed. Subsequently, a number of human cases of ehrlichiosis attributed to E. canis, or a very closely related ehrlichial agent as determined by morphologic and serologic examination, have been documented. However, a human isolate has not yet been obtained. This report describes the first in vitro propagation of virulent dog-derived E. canis in primary human monocyte cell cultures. A low passage of E. canis in primary canine monocytes was used as the inoculum for normal primary human monocyte cell cultures established 5 days previously. Initially, the infection rate of human monocytes was slow, with atypical, loosely packed morula formation being observed using light microscopy. As time in culture progressed, the percentage of infected cells increased significantly with E. canis morulae becoming more typical of those observed in cultured canine monocytes. These findings along with electron microscopic and serologic studies will be discussed in relation to the host-range and significance of this relatively newly recognized pathogenic characteristic of E. canis.

209 Human Rickettsia typhi infection in the Sinai, Egypt. R. Faris\*,  
M. Kenawi, G.A. Sattar, A.J. Saah, and A.F. Azad. Ain Shams University, Cairo, Egypt, Johns Hopkins School of Hygiene and Public Health, and University of Maryland, Baltimore, Maryland.

Serosurveys for R. typhi infection were done in Rafah and El Arish in the northeastern Sinai Peninsula from October to December 1987. Thirty-five males (median age 23, range 3 to 60 years) and 21 females (median age 38, range 4 to 60 years) were tested using a micro-IFA. Sera were available from local health clinics and hospitals; filter paper specimens were also collected at the same sites. Eight individuals were sick (5 with fever, 3 with diabetes mellitus), the remainder were well individuals.

Eight males (23%) and 5 females (25%) were positive by IFA. Three had reciprocal titers  $\geq 80$ , 7 were  $\geq 160$ , 1 was  $\geq 320$  and 1 was  $\geq 640$ . The age range was 8 to 60 years and was similar in both males and females.

Small rodents were trapped; ectoparasites and blood specimens were tested for R. typhi and for antibodies, respectively. These data and correlation with human infection will be presented.

- 210 THE DEVELOPMENT OF *ANAPLASMA MARGINALE* IN SALIVARY GLANDS OF *DERMACENTOR ANDERSONI*. K. M. Kocan\*, W. Goff, D. Stillier, A. F. Barbet, W. Edwards, S. A. Ewing, J. A. Hair, S. J. Barron, T. C. McGuire. Oklahoma State University, Stillwater, OK; USDA-ARS Animal Diseases Research Unit and Washington State University, Pullman, WA and University of Idaho, Moscow, ID; and University of Florida, Gainesville, FL.

Colonies of *Anaplasma marginale* have been identified in salivary glands of *Dermacentor* spp. but only in males held off the host and subjected to temperature change. Colonies have not been seen in feeding ticks, even transmitting ones. In this study salivary glands from males, infected as nymphs, were examined from (a) unfed ticks, not subjected to temperature change, (b) on each of 9 days of feeding; and (c) from ticks fed for 7 days, then held off the host for 15 days and subjected to temperature change. Assays included calf inoculation, light and electron microscopy, and a DNA probe. Homogenized salivary glands collected from unfed ticks did not cause infection when inoculated into a susceptible calf whereas those collected from feeding ticks (days 1-9) were infective. Parasite colonies were not seen in salivary glands of unfed ticks nor in those of feeding ticks, but colonies were seen in 15% of the ticks subjected to temperature change. The DNA probe identified *A. marginale* DNA in all groups; the probe signal increased as feeding progressed. The strongest probe signal was obtained from salivary glands of males subjected to temperature change. These data confirm the presence of *A. marginale* in salivary glands of feeding ticks and suggest that the parasite multiplies there. Failure to observe the parasite with light or electron microscopy in feeding ticks is puzzling but may have resulted from small numbers (the majority having been transmitted) or from its change to a cryptic form.

- 211 MAINTENANCE OF A PARALLEL TRANSMISSION CYCLE FOR *BORRELIA BURGENDORFERI* IN RABBITS BY *IXODES DENTATUS*. Sam R. TELFORD II\* and Andrew Spielman. Dept. Tropical Public Health, Harvard School of Public Health, Boston, MA USA.

Cottontail rabbits (*Sylvilagus floridanus*) serve as potential reservoir hosts for an enzootic cycle of transmission of the Lyme disease spirochete, *Borrelia burgdorferi*, because of their abundance, and the narrow host specificity of an *Ixodes* tick that may abundantly parasitize them. To test this suggestion of a cycle ancillary to that in *Peromyscus*, we evaluated the vector competence of *I. dentatus* for the spirochete, and examined the prevalence of infection in ticks and rabbits on Nantucket Island, Massachusetts. In the laboratory, 47% of nymphal *I. dentatus* became infected after feeding as larvae upon infected rabbits, as compared to 71% of *I. dammini* co-feeding on these hosts. The bite of a single nymphal *I. dentatus* was sufficient to transmit infection to a rabbit in one of two trials. The sera of 16 rabbits, collected on Nantucket, were tested by ELISA, and 56% were considered as reactive against antigens of *B. burgdorferi*. Of 30 unfed, nymphal *I. dentatus* swept from vegetation in the same site, 33% were infected by this spirochete, as determined by darkfield microscopy and by indirect immunofluorescence using monoclonal antibody H5332. Because infective nymphal and adult *I. dentatus* parasitize rabbits earlier in the transmission season than do nymphal *I. dammini*, and adult *I. dammini* seem never to feed on rabbits in nature, we suggest that rabbits may maintain a secondary cycle of this borreliosis, particularly in island locations where rabbit-feeding *Ixodes* are abundant. Thus, this borreliosis may be transmitted in silent foci devoid of such bridge vectors as *I. dammini*.

N: PARASITIC ENTOMOLOGY

212 THE EFFECT OF SPOOROZOITE DENSITY ON MALARIA TRANSMISSION BY  
PLASMODIUM FALCIPARUM- AND P. VIVAX-INFECTED ANOPHELES ALBIMANUS IN  
GUATEMALA.

R.F. Beach,\* C. Córdón-Rosales, and E. Molina. Medical Entomology  
Research and Training Unit/Guatemala, Division of Parasitic Diseases,  
Centers for Disease Control, Atlanta, GA.

An. albimanus, captured at a village on the Pacific Coast of  
Guatemala during February-March 1988, were analysed for P. vivax and P.  
falciparum sporozoites by ELISA. Fourteen of 2820 mosquitoes tested were  
positive for P. vivax sporozoite antigen. Only 2 females in the same sample  
were positive for P. falciparum sporozoite antigen. The mean sporozoite  
density for P. falciparum-infected females was 10 fold greater than the mean  
sporozoite density for the P. vivax-infected females. A blood smear survey  
at the end of the study detected 15 P. vivax and 15 P. falciparum infections  
among the 447 people living at the study site. None of the cases was  
imported. The ratio of P. falciparum sporozoite infections to P. vivax  
sporozoite infection, 1:7, was significantly less than the P. falciparum to  
P. vivax human case ratio, 1:1 ( $p < .002$ ). These results suggest that P.  
falciparum-infected An. albimanus may be more efficient vectors than P.  
vivax-infected females due to heavier sporozoite densities.

213 QUANTITATION OF MALARIA SPOOROZOITES TRANSMITTED IN VITRO  
DURING SALIVATION BY WILD NATURALLY INFECTED AFROTROPICAL  
ANOPHELES. J.C. Beier,\* F.K. Onyango, J.K. Koros, M.  
Ramadhan, R. Ogwang, C.M. Asiago, D.K. Koech and C.R. Roberts.  
Kenya Medical Research Institute and U.S. Army Medical Research  
Unit, Nairobi, Kenya.

Wild malaria-infected Anopheles mosquitoes from western  
Kenya were examined to quantitate sporozoites in the salivary  
glands and to evaluate transmission potential. The number of  
salivary gland sporozoites was determined microscopically for  
Anopheles gambiae s.l. (N=874, geometric mean (GM)=962, range:  
1-117,544) and An. funestus (N=263, GM=812, range:5-41,830). A  
total of 86.6% of 102 infected Anopheles transmitted sporozoites  
in vitro by salivation when their mouthparts were placed for 15  
min. into a capillary tube containing sucrose solution. The GM  
number of sporozoites transmitted was 3.86 for An. gambiae (N=80)  
and 3.59 for An. funestus (N=8) (range:1-34). Transmitting  
Anopheles contained significantly more salivary gland sporozoites  
(GM=1,136, N=88) than non-transmitters (GM=236, N=14).  
Sporozoites in the salivary duct of mosquito heads were detected  
microscopically in 80.3% (94/117) of Anopheles with salivary  
gland infections (GM=11.2, range:1-71). In nature, sporozoite  
transmission may be restricted to only sporozoites in the  
salivary duct at the time of feeding; transmitted sporozoites  
represented an average of only 0.4% of the total salivary gland  
sporozoites.

N: PARASITIC ENTOMOLOGY

- 214 A DIPSTICK, dot-ELISA, ASSAY FOR THE RAPID, FIELD IDENTIFICATION OF MOSQUITO BLOODMEAL SOURCES.

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A dot - enzyme linked immunoabsorbent assay employing strips of nitrocellulose paper, the dipstick, is presented. The assay is a modified indirect ELISA technique that allows for field identification of bloodmeal sources in less than two hours. Dipsticks inoculated with anti-human capture antibody may be blocked, dried and stored for future field use. No expensive laboratory equipment or training is required to conduct the assay, and all reagents are readily available from commercial sources.

- 215 LABORATORY AND FIELD MICROPLATE ASSAY DETECTION OF DDT RESISTANCE IN ANOPHELINES. W.G. Brogdon,\* R.F. Beach, J. Alarcon, A.M. Barber.  
Malaria Branch and Medical Entomology Research and Training  
Unit/Guatemala, CDC, Atlanta, GA; and Servicio Nacional de Erradicacion de la Malaria, Guayaquil, Ecuador, S.A.

A microplate assay-field photometer system has been developed for the detection of DDT resistance in mosquitoes. The assay detects resistance due to elevated glutathione s-transferase (DDT dehydrochlorinase). Single mosquitoes or pools of ten (blood-fed or not) were used in assays. Resistance levels are read spectrophotometrically using UV or visible wavelengths or are evaluated visually. Laboratory colonies of five species of the *Anopheles gambiae* complex and two strains of *An. albimanus* with resistance frequencies from 0 to 100% were used to validate the assay. Resistance is highest in colonized *An. arabiensis*, *An. quadriannulatus*, *An. merus* and *An. albimanus* from areas where DDT has been used for mosquito or agricultural pest control. Field data revealed varying levels of DDT resistance in Ecuadoran *An. albimanus*, *An. punctimacula* complex, and *An. trinkae* complex mosquitoes. Highest resistance (glutathione conjugation = 45 ng hr/mosquito) was found to occur in *An. trinkae* from east of the Andes (Amazon Basin) in an area where dieldrin was used as a herbicide. The DDT resistance detection method can be incorporated into a previously-developed system for detecting organophosphate, carbamate, and pyrethroid resistance. Supported by AID PASA LAC 0049-P-HC-6036.

## MONITORING ANOPHELES FREEBORNI LARVAL POPULATION DENSITIES IN CALIFORNIA RICE FIELDS USING REMOTE SENSING TECHNOLOGY

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A severe limitation in the efforts to control mosquito vectors of malaria is in the monitoring and predicting of vector abundance with any degree of precision. Currently, methods for monitoring larval mosquito populations are labor intensive, time consuming, and impractical. We report here the feasibility of using remote sensing technology to survey and predict the spatial distribution of larval *Anopheles freeborni*, the Western Malaria Mosquito, in commercial rice fields in northern California. During 1987, 104 rice fields were monitored for vegetation, water quality, and larval mosquito abundance. Remote sensing data (multispectral data in the visible and near-infrared regions of the electromagnetic spectrum) were acquired over these fields on ten dates from April 24 through August 6, 1987. *Anopheles freeborni* adult production begins to increase in late June, peaks in late August, and eventually ends in mid-September when the fields are drained in preparation for harvest. Estimates of total adult production on a field by field basis revealed that 36 out of the 104 fields produced 75% of the adult mosquitoes emerging from these fields. A discriminant function using the remotely sensed data was calculated to identify fields with high or low mosquito production. The percentage of high producing fields that were correctly classified ranged from 58% to 81%, the highest percentage occurring in mid-June, two months prior to peak adult production.

217 HEMOLYMPH PROTEIN ALTERATIONS IN MOSQUITOES DURING AN IMMUNE RESPONSE TO FILARIAL WORMS.

B.T. Beerntsen\* and B.M. Christensen. University of Wisconsin, Madison, WI.

Many studies have examined the immune response of *Aedes trivittatus* and *Aedes aegypti* Liverpool strain against intrathoracically inoculated filarial worms. It has been shown that *A. trivittatus* is significantly more immunocompetent than *A. aegypti*. Not only does *A. trivittatus* elicit a much stronger melanization response than *A. aegypti*, but distinct biochemical differences between the two species have been shown in monophenol oxidase activity in hemocytes and cell-free hemolymph. In the present studies, hemolymph, collected from mosquitoes of each species that had been intrathoracically inoculated with *Dirofilaria immitis*, was assayed for plasma protein alterations by gel electrophoresis. Differences were observed between plasma proteins of hemolymph from immune activated mosquitoes compared with hemolymph from saline inoculated and uninoculated mosquitoes. Differences also were noted in plasma proteins between the two species of mosquitoes. These data suggest that immune activation results in both quantitative and qualitative changes in the protein components of the plasma. Studies characterizing these different proteins are in progress. (Supported by NIH grant AI 19769.)

ASTMH POSTER SESSION II

- 219 ATTEMPTED IN VITRO CULTIVATION OF PLASMODIUM VIVAX USING HUMAN RETICULOCYTES. A.A. Adelugba, \* J.R. Murphy, V. Pawar, S. Baqar, and J. Davis. Program in Medical Technology and Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Md. 21201.

Plasmodium vivax - infected squirrel monkey erythrocytes (obtained from Dr. W. Collins, CDC, Atlanta) were introduced and admixed with suspensions of human erythrocytes which had been markedly enriched for reticulocytes and the resulting suspensions were maintained as in vitro cultures with or without further supplementation with reticulocytes. Parasites persisted for shorter intervals following introduction into cultures which did not contain reticulocyte enriched blood or into which reticulocyte enriched blood was introduced at less frequent intervals. The demonstration that P. vivax persisted best in those cultures containing the greatest number of reticulocytes is consistent with the view that reticulocytes are a needed substrate for in vitro P. vivax growth.

It is concluded that P. vivax was maintained in a reticulocyte enriched human blood based culture system under conditions which allowed about 2 complete asexual cycles to occur before parasites disappeared. It is further concluded that the most probable explanation for our failure to show a net increase in parasitemia resulted from the dilutions of cultures caused by the required continued addition of reticulocyte enriched blood.

- 220 Ca<sup>2+</sup>- and cAMP-DEPENDENT PROTEIN KINASES IN PLASMODIUM FALCIPARUM. L. K. Read\* and R. B. Mikkelsen. Department of Physiology, Tufts University, Boston, MA and Department of Radiation Oncology, Tufts-New-England Medical Center, Boston, MA.

Protein kinases activated by the second messengers Ca<sup>2+</sup> and cyclic adenosine 3',5'-monophosphate (cAMP) frequently play a role in the transmission of extracellular signals. The human malaria parasite, Plasmodium falciparum, undergoes processes normally controlled by such kinase-regulated pathways in other organisms. However, little is known about the presence of enzymes which would allow the parasites to respond to changes in second messenger concentrations. In this study, we have shown that erythrocytic stages of P. falciparum isolated by N<sub>2</sub> cavitation and Percoll gradient centrifugation contain both Ca<sup>2+</sup>- and cAMP- dependent kinase activities. Ca<sup>2+</sup>-dependent kinase activity was associated with the membrane fraction of isolated parasites, and was unaffected by phorbol esters, phosphatidylserine plus diacylglycerol, or calmodulin. Addition of  $\gamma$ -<sup>32</sup>P-ATP to membranes resulted in the Ca<sup>2+</sup>-dependent phosphorylation of proteins of molecular weights 195, 158, 127, 116, 85, 56, 51, 47.5, 21.5, and 21 kD. cAMP-dependent kinase activity was identified in the parasite cytosol both by cAMP-dependent phosphorylation of 195, 155, 80, 54, 51, 42, and 31.5 kD proteins and of exogenously added histone II-A. The kinase exhibited a K<sub>m</sub> for cAMP of 85 nM and an average V<sub>max</sub> of 131.3 pmol <sup>32</sup>P incorporated/min/mg protein. Phosphoamino acid analysis revealed both enzymes to be serine/threonine kinases. Demonstration of parasite protein kinases regulated by the second messengers Ca<sup>2+</sup> and cAMP provides a mechanism by which erythrocytic stages of P. falciparum can respond to changes in their environment.

ASTMH POSTER SESSION II

221 ROLE OF CYTOPLASMIC VESICLES IN P. FALCIPARUM INFECTED ERYTHROCYTES

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Intraerythrocytic development of the malarial parasite results in substantial changes of the host-cell membrane. Parasite encoded proteins are exported from the intracellular parasite and inserted into the host cell membrane. These proteins are transported through the parasite membrane, the parasitophorous-vacuole membrane and the erythrocyte cytoplasm. We have identified a P. falciparum encoded protein, a doublet of 50 kD / 45 kD (Pf 50), which is exported to the erythrocyte cytoplasm in parasite infected cells. Pf 50 is synthesized at early stages of intraerythrocytic development and persists until the parasite ruptures from infected cells. Based on its solubility properties, the protein was identified as an integral membrane protein. Immunofluorescence and immunoelectron microscopy demonstrated that Pf 50 is localized in the membrane of vesicles in the infected erythrocyte cytoplasm, vesicles which correspond to Maurer's Clefts. By immunofluorescence, Pf 50 was shown to colocalize with a P. falciparum soluble protein of Mr 130,000, which is secreted to the extracellular environment, and with actin which has a highly modified organization in the infected erythrocyte. Our data suggest that these vesicles are involved in protein trafficking across the erythrocyte cytoplasm.

222 HEREDITARY SPHEROCYTOSIS ASSOCIATED WITH SPECTRIN DEFICIENCY DOES NOT SUSTAIN THE GROWTH OF PLASMODIUM FALCIPARUM.

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To explore the role of red cell membrane proteins in the growth of P. falciparum we have studied parasite growth in the erythrocytes of 5 patients with hereditary spherocytosis (HS). HS spectrin deficient red cells are invaded normally but there is abnormal intraerythrocytic growth which is directly proportional to the spectrin deficiency. Red cells with a 30% decrease in spectrin sustained normal growth for the first two days but a significant decrease in the number of parasitized cells was observed by day 3 with further decreases subsequently. Similar results were obtained with red cells that had a 20% decrease in spectrin although the growth abnormality became apparent by day 4. Red cells that had smaller decreases (10%) in spectrin had correspondingly smaller decreases in parasite growth. Finally, HS red cells from a patient with normal amounts of spectrin had normal parasite growth, suggesting that other features of these spherocytic red cells were not playing a role in the decrease of growth. When parasitized spectrin-deficient HS red cells of day 4 were used as an inoculum for normal red cells, normal growth followed, suggesting that invasion by merozoites developed in HS cells was normal and that these merozoites were intrinsically viable as long as they did not have to grow in a spectrin-deficient host. These findings demonstrate that red cell membrane protein defects can result in intraerythrocytic growth abnormalities of P. falciparum.

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223 PLASMODIUM FALCIPARUM: OOKINETE PENETRATION AND OOCYST DEVELOPMENT IN ANOPHELINE MOSQUITOES.

J.F.G.M. Meis, G. Pool, G.J. v.Gemert, A.H.W. Lensen and T. Ponnudurai  
Department of Medical Parasitology, University of Nijmegen, The Netherlands.

It has been demonstrated in experimental P. bergeri infections that ookinetes follow an intracellular route. It is however, not known whether such a route damages host cells. It has also been shown that there is increased mortality of mosquitoes infected with this parasite about the same time that ookinetes are passing through the midgut wall. Recent work on P. falciparum infections, however, has pointed to the possibility of intercellular migration of the ookinete. Ultrastructural studies demonstrated different stages of intercellular localization of the parasite. Further evidence of intercellular migration of P. falciparum ookinete has been produced using cytochemical staining with ruthenium red. Intercellular migration of ookinetes does not damage cell membranes and we do not observe increased mortality of P. falciparum infected mosquitoes during this time even with very heavy parasite loads. The difference in mortality rate of P. bergeri and P. falciparum infected mosquitoes could therefore be related to the intra- or intercellular penetration of the ookinetes. More than 30 years ago, the avian parasite of P. gallinaceum has been described as penetrating the midgut epithelium of Aedes aegypti intercellularly (Stohler, Acta Trop 14, 303, 1957). It is therefore more than interesting, as it was shown recently that P. gallinaceum infected mosquitoes had no greater mortality than uninfected ones. (Freir and Friedman, J. Med. Entomol. 24,6, 1987). The similarity between the mammalian parasite P. falciparum and the avian one P. gallinaceum is very striking, demonstrating once again the "avian" nature of the former parasite. The further development of the P. falciparum oocyst beneath the basal lamina will be discussed.

224 P. falciparum associated placental pathology - a light and electron-microscopical and immunohistological study; Minoru Yamada, Richard Steketee, Matt Kida, Carlos Abramowsky, Jack Wirima, David Heymann, Joel Breman and Masamichi Aikawa, Case Western Reserve University, Cleveland, Ohio, Centers for Disease Control, Atlanta, Georgia and Ministry of Health, Malawi.

Pathological changes were studied by light and electron microscopy on 18 placentas collected from postnatal women (7 primiparas and 11 multiparas) in Malawi infected with P. falciparum. Two placentas from uninfected primiparas were studied as a control. Pathological changes included the presence of parasitized erythrocytes in the intervillous space, malarial pigment deposits in trophoblasts or free in the intervillous space, trophoblastic damage with increased fibers, and partial loss of microvilli and thickening of the trophoblastic basal membrane. No infected erythrocytes were seen in the fetal circulation. Immunohistological examination of the placentas with a rabbit antiserum against P. falciparum revealed positive staining in the cytoplasm of trophoblasts. Severity of the pathology appeared to correlate with the level of parasitemia in the placentas. There was no marked difference in the severity of pathological changes in primiparas versus multiparas. No correlation was found between the severity of pathological changes and infant birth weight or placental weight.

ASTMH POSTER SESSION II

225 CHARACTERIZATION OF A MODEL OF PREGNANCY-ASSOCIATED MALARIAL PATHO-  
PHYSIOLOGY: PLASMODIUM BERGHEI IN THE WHITE RAT

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Both the pregnant mother and her fetus are affected to an extraordinary degree by Plasmodium falciparum malaria. The underlying cause(s) of this enhanced virulence is imperfectly understood, one reason being that research on the problem has been hampered by lack of a suitable, well-characterized model. The mouse-P. berghei system is not satisfactory because of the invariably fulminant infection in both pregnant and non-pregnant mice. The white rat with its age-related immunological ability to resolve the infection may be of greater potential as a model of pregnancy-associated malarial pathophysiology. We have characterized this model system and identified some of the factors affecting the course of infection, morbidity and mortality. The course of parasitemia and anemia (hematocrit) was followed in pregnant rats and age-matched female non-pregnant controls. The effects of age, stage of pregnancy at initiation of infection, and primiparity compared to multiparity (factors reported to affect the degree of pathogenicity in the pregnant human) were determined. Under all experimental conditions the parasitemia was higher and the anemia more profound and prolonged in the pregnant animals than in the controls. The infection was most severe, being almost always fatal, when it was initiated at the second week of pregnancy and attaining peak parasitemia near, or at, term. Primigravids had, as a group, more severe infections than multiparous rats. Comparison of the parasitemia in maternal peripheral blood at time of delivery and in placental blood revealed a deep vascular sequestration and schizogony in the placenta, particularly immediately before and after parasitemic crisis.

226 COMPARISON OF THE BIOLOGIC ACTIVITY OF ANTIBODIES SPECIFIC TO A  
PLASMODIUM FALCIPARUM 83 KD EXOANTIGEN AND A SYNTHETIC PEPTIDE  
DERIVATIVE USING AN IN VITRO NEUTRALIZATION ASSAY.

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In vitro neutralization assays for Plasmodium falciparum have greatly facilitated screening of potentially protective antigens by assaying the biologic activity of immune serum specific to these antigens. An in vitro neutralization assay has been used to analyze the biologic activity of rabbit antibodies specific to an 83 KD exoantigen purified from supernatant fluids of P. falciparum Geneve/SGE-1-infected cultures. Rabbit antisera to a synthetic 29-amino acid peptide derived from the 83 KD N-terminus, analyzed for P. falciparum-specific growth inhibition, resulted in 46% inhibition after 72 hrs in culture, as compared to 51% obtained with antisera to the parent polypeptide. The specificity of anti-29mer immunoglobulins was elucidated by pre-incubation of IgG with the 29mer peptide, which abolished its specific growth inhibitory capacity. Furthermore, use of rabbit anti-29mer IgG, affinity-purified with the 29mer peptide, resulted in 47% inhibition at a concentration of 0.1 mg/ml after 72 hrs in culture, as opposed to 36-48% obtained with non-affinity-purified rabbit anti-29mer IgG at a concentration of 1 mg/ml. Use of these 29mer-specific antibodies in in vitro neutralization assays has identified their P. falciparum-specific growth inhibitory capacity and suggest a protective potential for the 29mer peptide in vivo, as well as supporting the use of synthetic immunogens as vaccines.

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227 A STRUCTURE-FUNCTION MODEL FOR PLASMODIUM FALCIPARUM 175-KD  
ERYTHROCYTE BINDING ANTIGEN.

J. David Haynes,\* Palmer A. Orlandi, Michael Zegans, Francis W. Klotz,  
and Jeffrey D. Chulay. Department of Immunology, Walter Reed Army Institute of  
Research, Washington, DC 20307.

A soluble 175kD erythrocyte binding antigen (EBA-175) binds to both merozoites and erythrocytes in ways that correlate with invasion (Camus and Hadley, 1985. Science. 230:553). It is now proposed that not merely the binding but also the configuration of the bound EBA-175 is important for biologic activity correlating with invasion. From experiments with or without inhibitors of endogenous erythrocyte proteases, it appears that a previously described 65kD EBA is a proteolytic fragment of EBA-175. Most erythrocytes that are not invaded by P. falciparum (e.g., guinea pig, rabbit, and neuraminidase-treated-human erythrocytes) bind neither EBA-175 nor its 65kD fragment. Rhesus erythrocytes are not invaded by P. falciparum and were originally described as binding large amounts of the 65kD EBA but not binding EBA-175. It is now shown that rhesus erythrocytes bind EBA-175 (in the presence of protease inhibitors) but that EBA-175 is readily cleaved to 65kD that remains bound (in the absence of protease inhibitors). In semi-quantitative studies comparing binding to human erythrocytes, more EBA-175 bound and bound more tightly to rhesus erythrocytes. Perhaps when EBA-175 is bound to rhesus erythrocytes, it assumes an abnormal configuration that is both more open to proteolysis and less suitable for its normal functioning in invasion. This is similar to the findings of Camus and Hadley that EBA-175 of one strain bound to merozoites of an incompatible strain but was degraded to 165kD and did not function well in invasion. Exogenous proteases have been used also to gather data that support a 4-domain, structure-function model of EBA-175.

228 IDENTIFICATION OF A RHOPTRY-ASSOCIATED ANTIGEN OF THE SIMIAN MALARIA  
PARASITE PLASMODIUM FRAGILE WITH MONOCLONAL ANTIBODIES. P. M.

Procell,\* P. Deloron, W. E. Collins, and P. Nguyen-Dinh.  
Malaria Branch, CDC, Atlanta, GA.

Plasmodium fragile is a natural parasite of macaques which produces an infection closely resembling P. falciparum in humans. Similarities between host-parasite interactions of P. falciparum in humans and P. fragile in rhesus monkeys are currently being studied. To identify P. fragile antigens which may be analogous to already defined antigens of P. falciparum, monoclonal antibodies (MAbs) were produced against the asexual blood stage parasites of P. fragile. The fusion of immune mouse spleen cells with the myeloma cell line SP2/0 resulted in four MAb-secreting cloned hybridoma cell lines. One MAb was an IgG1, one IgG2a, and two IgG3. When tested in an indirect immunofluorescent antibody (IFA) assay with P. fragile, all of the MAbs produced a rhoptry-associated IFA pattern previously described with P. falciparum antibodies. This reactivity was associated with mature schizonts and free merozoites. None of the MAbs cross-reacted with the asexual blood stages of P. falciparum (HB3 strain) when tested in the IFA assay. Using Western immunoblot one of the MAbs identified a polypeptide of  $M_r$  125,000. Identification with MAbs of these and other P. fragile antigens, which may be functionally analogous to P. falciparum antigens, constitutes an initial step in the study of defined antigens during infection in the natural host. Supported in part by USAID PASA BST-0453-P-HC-2086-03.

ASTMH POSTER SESSION II

229 IMMUNOGENICITY OF NATIVE P. FALCIPARUM GP195 IN FCA VERSUS THE COMBINATION OF B30-MDP, LA-15-PH, and TDM IMMUNOMODULATORS.

L.Q. Tam\*, G.S.N. Hui, S. Kotani, T. Shiba, S. Kusumoto, and W.A. Siddiqui. Dept. of Tropical Medicine, Univ. of Hawaii, Honolulu, HI 96816 and Osaka College of Medical Technology, Osaka, Japan 530.

We have shown that the P. falciparum major merozoite surface precursor proteins (gp195) can completely protect Aotus karyotype II/III monkeys against lethal challenge infection, when given with Freund's complete adjuvant. In the development of an asexual blood stage vaccine based on gp195, it is important to demonstrate a safe adjuvant that can effectively replace FCA. However, we have found that native gp195 given with a single immunomodulator, MDP(Lys)L18 emulsified in intralipid, does not induce protective immunity. Therefore, we evaluated the immunogenicity of native gp195 given with the combination of three immunomodulators. B30-MDP is a lipophilic derivative of muramyl dipeptide undergoing clinical evaluation. LA-15-PH is a synthetic lipid A analog, corresponding to the major structure of non-toxic monophosphoryl lipid A of RIBI Immunochem. Trehalose dimycolate (TDM) is a cell wall component of the Corynebacteria, Nocardia, Mycobacterium group shown to act synergistically with monophosphoryl lipid A by RIBI Immunochem. Rabbits were immunized with 50 ug of gp195 in FCA or the combination of the three immunomodulators emulsified in squalane oil or incorporated in liposomes. It was found that the three immunomodulators presented in liposomes but not in squalane oil produced anti-gp195 responses nearly comparable to levels induced by FCA. The data indicate that this formulation may be capable of replacing FCA in monkey vaccination experiments and efficacious in humans. (Supported by USAID)

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ALBUMIN BINDING COMPONENTS OF PLASMODIUM BERGHEI: NATURE OF THE COMPONENTS, THEIR INTERACTION WITH ALBUMIN AND WITH ANTIPLASMODIAL ANTIBODY.

G. Green IV,\* and J.P. Kreier. The Ohio State University, Columbus, OH.

We have previously reported that some components of p. berghei bind to albumin. The nature of these materials and of the interaction with albumin has been further characterized. The components of a soluble preparation of whole infected red blood cells which bound to albumin were collected by affinity chromatography. The materials were studied by SDS-PAGE protein immunoblotting and ELISA.

The amount of plasmodial materials required to saturate the albumin on the columns was studied by varying the amount of sample loaded into standard affinity columns containing 200 mg bound albumin. Samples of a solubilized IRBC preparation containing from 0 to 400 mg of protein were loaded on the columns. The columns became saturated with sample of 150 mg protein. At saturation, yield was 70.4 µg of eluate, a yield of 0.032%. With larger sample sizes, yields were identical.

The major polypeptides detected in silver stained PAGE gels were two with molecular weights of about 52,000 D. A single polypeptide of 90,000 D, two of 70,000 D, two of 52,000 D and one of 31,000 D were the major polypeptides detected by the immunoblotting technique. Protein immunoblots of the eluate which had been stored for several days lacked the polypeptides of 70,000 D and accumulated breakdown products of 32,000 to 48,000 D, suggesting that proteolysis results in production of small immunogenic polypeptides.

Inhibition by albumin of binding to plasmodial antigens of antibody in serum from rats immunized with the eluate from the albumin affinity columns occurred. The presence of rat albumin at a concentration of 1 mg/ml in the ELISA test reduced titers of the immune serum from 640 to 80. Increased concentrations of albumin further reduced titers. In comparison, the presence of hemoglobin at 1 mg/ml did not reduce the titer of the serum. Albumin had less effect on antiplasmodial titers of hyperimmune serum than on serum raised to the eluate; at 1 mg/ml the reduction of the titer of the former sample was only from 1280 to 640.

The albumin binding components' reaction with albumin was saturable suggesting specificity. Western blotting showed the materials in the eluate were of parasite origin. The inhibition by albumin of the antigen-antibody reaction suggests that albumin may compete with antibody for reaction with plasmodial antigens and thus be involved in the survival of Plasmodia in the host. We are at present attempting to detect the location of the albumin binding component in the parasite by immunoelectron microscopy. If these components should be on the surface of merozoites, they could have a role in the development of an antigenic disguise.

ASTMH POSTER SESSION II

- 231 INFECTION WITH PLASMODIUM MALARIAE ELICITS ANTIBODIES WHICH REACT WITH PLASMODIUM BRASILIANUM-INFECTED ERYTHROCYTE MEMBRANES. A.J. Sulzer,\* R.A. Cantella, and P. Millet. Malaria Branch, CDC, Atlanta, GA; and Universidad Peruana Cayetano Heredia, Lima, Peru.

Sera from P. malariae infections were compared for their antibody reactivity with P. brasilianum. Standard IFA (SIFA) was used to determine reactivity to whole parasites, and modified IFA (MIFA) was used to determine reactivity with glutaraldehyde-fixed and air-dried infected erythrocytes. During the course of infection with P. malariae, MIFA antibody reactivity was found in sera from 3 of 5 chimpanzees with membranes of both ring- and mature-stage P. brasilianum-infected erythrocytes. This reactivity was then examined in sera from two villages in Peru, endemic for P. vivax and P. malariae. At Cheni, where P. malariae was recently introduced, 72% of 60 sera reacted in SIFA (GMT, 1:2,195). Only 30% reacted with membrane-associated antigens of P. brasilianum (GMT, 1:324). The GMT of SIFA of these 18 sera was 1:9,541. At Cutiverini, endemic for P. malariae for many years, 84% of 106 sera reacted in SIFA (GMT, 1:2,778). 65% of the sera reacted with parasitized erythrocyte membranes (GMT, 1:1389). The GMT of SIFA titers of these 69 sera was 1:2,896. The longer endemicity of P. malariae at Cutiverini resulted in the more frequent presence and greater titers of membrane-reactive antibody. The ratio of membrane-reactive antibody GMT to the SIFA titer GMT was also higher in Cutiverini than in Cheni. Further characterization of the distinction between reactivity to membranes of ring stage versus mature stage parasites will be presented. Supported in part by USAID PASA BST-0453-P-HC-2086-03.

- 232 CHARACTERIZATION OF A 50 KDA ANTIGEN FOUND IN IMMUNE CLUSTERS OF PLASMODIUM FALCIPARUM MEROZOITES. A.W. THOMAS,\* D.A. CARR, J.A. LYON and J.D. CHULAY. Dept. Immunology, Walter Reed Army Institute of Research, Washington DC 20307-5100.

When P. falciparum schizont-infected erythrocytes are cultured in the presence of immune serum, antibodies interfere with the merozoite disaggregation that occurs during rupture, immune clusters of merozoites (ICM) form, and parasite growth is inhibited. Because antibodies present in immune complexes generated during ICM formation appear to play a role in growth inhibition and protection against parasites, the target antigens for these antibodies may be useful for vaccine development. Antibodies dissociated from ICM have been used to probe 2D Western blots of P. falciparum schizont antigens. One of the antigens recognized by antibody eluted from ICM is a 50 kDa acidic protein. We have isolated from a lambda gt11 genomic DNA expression library a clone containing a 2.4 kb insert which codes for a fusion protein that selects antibody specific for the 50 kDa antigen from polyclonal, polyspecific antiserum. The DNA sequence of this clone has been established. A 945 bp open reading frame, in frame with beta-galactosidase, encodes a predicted 70% of the C-terminal end of the protein. Peptides have been synthesized according to the predicted amino acid sequence. Antibodies raised against these peptides will enable further characterization of this antigen as a vaccine candidate.

ASTMH POSTER SESSION II

- 233 IMMUNOGENICITY OF A CARRIER-FREE SYNTHETIC PEPTIDE COMPLEX DERIVED FROM A 70 KD PLASMODIUM FALCIPARUM EXOANTIGEN.  
M.A. James,\* S. Montenegro-James, C.J. Fajfar-Whetstone, S.-J. Ma, and M. Ristic. Department of Veterinary Pathobiology, Univ. of Illinois, Urbana, IL

We have previously reported the immunogenicity and induction of partial protection against virulent challenge exposure in squirrel monkeys vaccinated with a single 70 kd P. falciparum schizont exoantigen. Recently, internal chymotryptic peptide digests of the 70 kd protein have been prepared and amino-acid sequences determined. Four peptides (C2,C3=29mer,C5,C10) were subsequently selected for synthesis, based on their predictability for antigenic sites. The antigenicity of the synthetic peptides was confirmed by the high degree of reactivity with sera from individuals residing in malaria-endemic regions of Latin America, Africa and S.E. Asia. In the present study, we showed that the above peptides could be conjugated with glutaraldehyde in the absence of a carrier and used successfully as an immunogen. Two rabbits were given a series of immunizations and isotype-specific antibody responses analyzed by the IFA test, peptide-EIA and Western immunoblots. High levels of schizont-specific antibody were obtained with IFA titers of 1:160 observed post-second immunization. Moreover, these antisera reacted predominantly with a major 70kd protein in supernatant fluids and in infected erythrocyte lysates. High IgG titers to the synthetic peptide complex demonstrate the induction of T cell-dependent antibody responses, suggesting the presence of peptide-specific T cell epitopes, and indicate the potential use of synthetic peptide complexes as immunogens unmodified by carrier conjugation.

- 234 THE APPEARANCE AND FATE OF THREE DIFFERENT SPOOROZOITE ANTIGENS OF PLASMODIUM YOELLI TRACKED BY IMMUNOELECTRON MICROSCOPY.  
R.L. Beaudoin,\* C. Atkinson, L.A. Beaudoin, M. Sedegah Y. Charoenvit and M. Aikawa.  
N.M.R.I., Bethesda, MD, Case Western Reserve University, Cleveland, OH and PAHO, Washington, DC.

Monoclonal antibodies (Mabs) directed against sporozoites of Plasmodium yoelli recognize at least two antigens in addition to the circumsporozoite (CS) protein. Although their function is yet to be clarified, these antigens may eventually be important in vaccine development. In the present study appearance and distribution of each of these stage specific antigens was determined by immunoelectron microscopy using colloidal gold to detect Mabs bound to antigens in the sporozoite. Antigenic determinants recognized by 5 Mabs were studied. Mabs NYS1, 2 and 3 recognize the CS antigen, although each reacts with a different epitope. This antigen can be detected with each of these 3 Mabs as early as 5 days after initiation of the sporogonic cycle. Gold particles can be seen associated with the endoplasmic reticulum (ER) of this early oocyst until clefts begin to appear at which point the particles concentrate on thickened surfaces of the plasma membrane lining the germinal clefts. However, particle distribution on mature salivary gland sporozoites differs with the Mab used. The remaining Mabs recognize 2 non-CS antigens. One of these is a 140 kDa protein which a common determinant with the CS antigen. The second non-CS antigen could not be found by Western blotting, however both of the genes encoding these 2 antigens have been cloned and are being sequenced. The 2 non-CS antigens also appear early in the sporogonic cycle and gold particles can be found internally at 5 days although they are sparse. Once sporozoite formation begins however, gold particles locating the NYS5 antigen become abundant, especially in the budding sporozoite. Both these antigens appear to be associated with micronemes although gold particles were not seen on rhoptries indicating that more than 1 species of microneme may be present. In the mature sporozoite the gold particles labelling the NYS4 antigen were clustered often close to the surface and external clusters were also seen suggesting that this may be a secretory antigen. All 3 antigens are shed as shown by the substantial numbers of particles seen free of sporozoites.

ASTMH POSTER SESSION II

235 PRESENTATION OF A SYNTHETIC PLASMODIUM BERGHEI CIRCUMSPOROZOITE PEPTIDE CONJUGATE ON THE SURFACE OF  $G_{M1}$ -BEARING LIPOSOMES.

L.D. Loomis, R.L. Richards, J.C. Sadoff, C.R. Alving, W.T. Hockmeyer and W.R. Ballou. Departments of Immunology and Membrane Biochemistry, Walter Reed Army Institute of Research, Washington D.C.

Antigen presentation is known to be important in the induction of protective immune responses against malaria sporozoites. We prepared a synthetic conjugate vaccine containing Plasmodium berghei circumsporozoite (CS) repeat peptide (DPAPPNAN)<sub>3</sub> (24mer) covalently coupled to cholera toxin protein (CG), the B subunit of cholera toxin. The conjugate (24merCG) was irreversibly bound to liposomes bearing ganglioside  $G_{M1}$  on their surfaces by way of the  $G_{M1}$  binding site on CG. C57Bl/6 mice were immunized with 10 ug doses (based on 24mer) i.v. or i.p. at weeks 0, 3, and 7. Control groups received 24merCG alone or in Freund's adjuvant i.m., or empty liposomes (no  $G_{M1}$  or 24merCG) i.v. or i.p. Mice immunized with {Lip( $G_{M1}$ )+24merCG} developed antibody titers comparable to those immunized with 24merCG in Freund's and were significantly protected against low dose P. berghei sporozoite challenge. These studies demonstrate the feasibility of constructing highly immunogenic synthetic peptide-based subunit vaccines that exploit the unique properties of liposomes and  $G_{M1}$ -CG interactions.

236 RESTRICTION OF MURINE T CELL REPERTOIRES TO PLASMODIUM BERGHEI CIRCUMSPOROZOITE PROTEIN AS A FUNCTION OF ANTIGEN PROCESSING.

U. Krzych\*, H. Link, T. Jareed, W. R. Ballou. Dept. of Biology, Catholic University of America, and Dept. of Immunology, Walter Reed Army Institute of Research, Washington, DC.

In numerous host species the antibody response to Plasmodia sporozoites has been shown to be almost exclusively directed against immunodominant repeat epitopes on the circumsporozoite (CS) protein, and these B cell epitopes form the basis of sporozoite vaccines now in clinical trials. Because an optimal subunit sporozoite vaccine should contain epitopes that induce both B and T cell responses, we explored the T cell repertoire for specificities directed against determinants on the CS protein in mouse strains that can be readily protected by immunization with irradiated P. berghei sporozoites. Spleen cell populations from sporozoite-immunized Balb/c, C3H/HeN, and C57Bl/6 were assayed for proliferative responses to irradiated sporozoites or synthetic peptides representing nearly the complete CS protein. Highly restricted responses to synthetic peptides were observed that contrasted with uniform responsiveness to whole sporozoites. Similarly restricted, strain-specific, anti-peptide responses were observed with draining lymph node populations obtained from mice immunized with sporozoites in complete Freund's adjuvant (CFA). In contrast, mice primed with a full length recombinant CS protein (PbCS) in CFA developed T cell specificities not seen after sporozoite priming, and PbCS-primed cells were unresponsive to irradiated sporozoites. These data suggest that the expression of the T cell repertoire for CS antigens depends upon the molecular context of the priming antigen which determines a particular pathway of antigen processing and presentation. Consequently, priming with irradiated sporozoites may result in a display of CS protein-specific T cell epitopes unique for that antigen, and different from PbCS. These findings are particularly relevant to the development of malaria vaccines designed to induce protective T cell responses.

ASTMH POSTER SESSION II

237 PREVALENCE OF CIRCUMSPOROZOITE ANTIBODIES TO PLASMODIUM VIVAX IN PENINSULAR MALAYSIA: HUMAN IMMUNE SERA REACT WITH NS1<sub>81</sub>V20 A RECOMBINANT VIVAX SPOROZOITE VACCINE CANDIDATE.

M. Lee\*, D. R. Davis, W. R. Ballou, G.F. Wasserman, and G.E. Lewis Jr. U.S. Army Medical Research Unit-Malaysia, Institute for Medical Research, Kuala Lumpur and Department of Immunology, Walter Reed Army Institute of Research, Washington, D.C. and Department of Protein Biochemistry, Smith, Klein and French Labs, Swedeland, PA.

Malarimetric studies conducted in the Pos Legap area of Peninsular Malaysia revealed intense year-round malaria transmission and a consistent malaria point prevalence of 38-40% (P.v 29%, P.f 27%, P.m 8% and mixed infection 37%) among an indigenous Orang Asli population. If life-long inoculation with sporozoites by natural means, i.e. mosquitoes, induces circumsporozoite (CS) protein specific antibodies, then such antibodies should be present and readily measurable in this population. Sera from 94 of 595 individuals surveyed were selected at random and reactivity to a recombinant vivax sporozoite vaccine candidate, NS1<sub>81</sub>V20, was measured by ELISA. CS antibodies were present in 72% of the sera tested. Sera from no less than 53% of the subjects in each of 6 age groups were reactive. ELISA results correlated well with IFA (intact P. vivax sporozoites served as the antigen). These data indicate that the immunodominant CS epitopes of P. vivax sporozoites are highly immunogenic for humans and suggest that NS1<sub>81</sub>V20 is a suitable vaccine candidate.

238 A MODEL FOR DETAILED STUDIES ON THE SPOROGONY OF MALARIA. V.E. Do Rosario, R. Coleman, P. Leland, M. Hollingdale, A. Appiah and J. Vaughan \*.

\*. Biomedical Research Institute, Rockville MD; Dept. Entomology, WRAIR, Washington DC; , University Maryland Baltimore MD.

In order to conduct studies such as vector species refractoriness or effects of drugs or antibodies on sporogony, precise monitoring of the sporogonic cycle is required. Previous studies have been limited to oocyst counts and salivary glands indices on few selected days of the cycle. Here we describe a methodology to effectively partition the different parasite growth stages that can occur concurrently within an individual mosquito (i.e., oocyst, hemocoel sporozoites, salivary gland sporozoite). Each mosquito was processed as follows: hemolymph was collected from the thorax with a fine-tipped microcapillary tube and spotted onto a slide. The sample was methanol fixed, stained using IFA techniques and examined for sporozoites with U.V. microscopy. Following collection of hemolymph, salivary glands were excised and examined under phase contrast microscopy for sporozoites. Lastly, midguts were excised, stained with mercuro-chrome and examined for oocysts. This procedure was performed on a daily basis on pools of mosquitoes to accurately monitor the course of infection. Using a team of 4 people, we were able to process 75 mosquitoes in 2 hours. This model has allowed us to study a) the effect of mefloquine, chloroquine and qinghaosu on Plasmodium berghei infected Anopheles stephensi mosquitoes, b) passage and attachment of anti-sporozoite antibody onto hemocoel sporozoites, c) comparative dynamics of the sporogonic cycle of P.berghei ANKA parasites in both A. stephensi and A. freeborni mosquitoes. This is work was supported by WHO Grant 870105 (VER), NIH AI-17828 (JAV), and by a National Research Council Fellowship to REC.

ASTMH POSTER SESSION II

- 239 ULTRASTRUCTURAL LOCALIZATION OF CIRCUMSPOROZOITE PROTEIN IN P. CYNOMOLGI EXOERYTHROCYTIC SCHIZONTS  
Carter T. Atkinson,\* Pascal Millet, Alan Cochrane, William E. Collins and Masamichi Aikawa, Institute of Pathology, Case Western Reserve University, Cleveland, Ohio and Malaria Branch, Centers for Disease Control, Atlanta, GA New York University, New York, NY

In spite of the importance of the circumsporozoite (CS) protein of malarial parasites in vaccine development, little is known about its fate after sporozoites invade hepatocytes. We examined the distribution of CS protein in *in vitro* cultures of *P. cynomolgi* exoerythrocytic (EE) schizonts by immunoelectron microscopy with monoclonal antibodies and protein A-gold. Dense label was associated with the surface, parasitophorous vacuole (PV) space and PV membrane surrounding immature *P. cynomolgi* EE schizonts, but became patchy and sparsely distributed over the surface of 5 day schizonts. Little label was associated with mature 7 and 8 day schizonts. This study demonstrates persistence of CS antigen in early immature EE schizonts, suggesting that sporozoite vaccines may have an effect on this stage of development.

- 240 ADOPTIVE TRANSFER OF PROTECTIVE IMMUNITY TO PLASMODIUM YOELII SPOOROZOITES.  
S.J. Pancake,\* Y. Charoenvit, M. Sedegah, R.L. Beaudoin, and S.L. Hoffman. Infectious Disease Department, Naval Medical Research Institute, Bethesda, MD.

The effector arm of the protective immune response induced by immunization of Balb/c mice with irradiated *Plasmodium yoelii* sporozoites requires CD8+ lymphocytes, although the antigens involved in this immunity are currently unknown. In order to study this response experimentally, we have standardized a protocol for adoptively transferring protection based on the original system for *P. berghei* malaria. Spleen cells from Balb/c mice immunized with 3 doses of irradiation attenuated *P. yoelii* sporozoites were isolated and transferred into irradiated (500 rads), otherwise naive mice. Twenty four hours later all recipients were boosted with  $10^4$  irradiated *P. yoelii* sporozoites, and 7 days later they were challenged with 200 infective sporozoites. In the *P. berghei* system, transfer of only  $1.5 \times 10^6$  immune spleen cells are required to achieve 80-100% protection against a  $10^4$  sporozoite challenge. In contrast, in the *P. yoelii* system at least  $5 \times 10^7$  immune spleen cells are required to achieve 67%-100% protection against only a 200 sporozoite challenge. The system will be used to further characterize protective immunity against the pre-erythrocytic stages of *P. yoelii*.

ASTMH POSTER SESSION II

- 241 CIRCUMSPOROZOITE PROTEINS OF MALARIA SPOROZOITES APPEAR TO BE SECRETORY PROTEINS. Michael J. Stewart\*, Alan H. Cochrane, and Jerome P. Vanderberg. Department of Medical & Molecular Parasitology, New York University School of Medicine, New York, NY 10016.

We recently reported that gliding malaria sporozoites leave behind trails of circumsporozoite (CS) protein that correspond to their patterns of movement (Stewart & Vanderberg; *J. Protozool.*, in press). Using a two-site/one-antibody immunoradiometric assay to detect CS protein, we now further confirm the release of CS protein by demonstrating that CS protein is apparently secreted from freshly-isolated *P. berghei* sporozoites into the medium, while the amount of CS protein from intact sporozoites remains relatively constant over the time period of the experiment. In some cases, the amount of released CS protein over time is greater than total CS protein at time zero. This phenomenon is dependent on the following parameters: time, temperature, and batch of sporozoites (some batches are more viable than others). However, secretion is not directly dependent upon motility. The results of Western blotting, metabolic labeling and immunoprecipitation, metabolic inhibitor studies, and other studies used to further characterize secretion and to identify the component CS proteins involved, will be discussed. These findings prompt us to reconsider how the CS protein is associated with the sporozoite surface.

Supported by NIH Grant AI 24615-01A1.

- 242 Progress in the Control of Malaria in Zimbabwe. P. Taylor. Blair Research Laboratory, Causeway, ZIMBABWE.

Malaria control programmes started in the late 1940's in north-east Zimbabwe. The progress since this time has been reviewed together with data on the impact of this control on the transmission of malaria. Prevalence data show a dramatic fall in the few areas for which there are data and five years of malaria control reduced malaria prevalence from 40% to 1%. The efficacy of DDT for vector control is supported.

The lack of a good health information system precludes comparisons of past and present incidence of the disease but recent data show that clinical malaria is unreliable and unsuitable for planning. Data from 16 rural clinics showed that almost 10% of outpatients were treated for malaria but that only 10% of these had a positive blood slide.

The present malaria situation is discussed with emphasis on the rapid deterioration expected in most unstable malaria areas of Africa in the future.

ASTMH POSTER SESSION II

- 243 SPECIFIC, NON-ISOTOPIC DETECTION OF PLASMODIUM FALCIPARUM IN BLOOD SMEARS USING ENZYME-LINKED SYNTHETIC DNA.  
 J.E. Marich<sup>\*,1</sup>, J.L. Ruth<sup>1</sup>, C.J.T.F. Whetstone<sup>2</sup>, W.E. Collins<sup>3</sup> and G. L. McLaughlin<sup>2</sup>. <sup>1</sup>Molecular Biosystems Inc., San Diego, <sup>2</sup>University of Illinois, Urbana and <sup>3</sup>Centers for Disease Control, Atlanta.

Light microscopic examination of malaria in Geimsa-stained slides can be problematic. Mixed infections and polymorphisms due to drug therapies or immune responses can make parasitemia determination and speciation difficult. Using an alkaline phosphatase conjugated oligonucleotide probe (PFR1-AP) specific for P. falciparum (1987, The Lancet 1: 714-6), a rapid nucleic acid in situ hybridization assay was developed for the detection of this pathogen in blood smears. Examined under oil immersion microscopy, parasite nuclei and cytoplasm were stained. Trophozoites and schizonts stained more intensely than ring stages. In blind experiments, P. falciparum parasitemias from culture and infected Actus blood matched those determined in duplicate Geimsa-stained smears. P. vivax and P. brasilianum infected cells from Actus and Saimuri infections as well as white cells were not stained by the probe. Hybridization, washing and detection do not require temperature control and can be performed from 20 to 40°C. The assay is rapid and easy. Samples can be performed in batch with 30 to 60 smears processed simultaneously at a total processing time of 3 hours. This assay provides a simple, specific and sensitive method for the diagnosis of P. falciparum in blood smears. Supported by DiaTech Sub-agreement No. 861100160 (G.L.M. and J.L.R.).

- 244 PROGNOSTIC INDICATORS IN PEDIATRIC CEREBRAL MALARIA.  
 T.E. Taylor\*, M.E. Molyneux, Michigan State University College of Osteopathic Medicine, East Lansing, MI, The Liverpool School of Tropical Medicine, Liverpool, UK.

P. falciparum malaria is a major cause of childhood morbidity and mortality in sub-Saharan Africa. Correct treatment requires prompt diagnosis and assessment of severity. A prospective study of 180 children (age <12 yrs.) presenting to the Queen Elizabeth Central Hospital, Blantyre, Malawi, with altered consciousness and asexual P. falciparum parasitemia was conducted to identify admission clinical features which are prognostic indicators of an adverse outcome. We evaluated admission history, laboratory data and physical findings, including a modification of the Glasgow Coma Scale (modified for use with preverbal and verbal children) to determine predictors of death or neurologic sequelae. Children presenting with hypoglycemia, convulsions, age < 3, or a coma score = 0 (unresponsive to painful stimuli) were more likely to die or develop neurologic sequelae (see table). Raised concentrations of plasma and CSF lactate were associated with the patient's glycemic status and were not of independent prognostic value. Adoption of this modified coma scale, and consideration of the prognostic indicators described would facilitate the early recognition of children at increased risk of adverse outcomes; in addition, standardization of the admitting evaluation would enhance the comparability of clinical malaria studies.

Feature	Relative risk of:	
	Death	Death or sequelae
Coma score = 0 on admission	18	4.3
Hypoglycemia (blood glucose < 2.2mmol/l)	9.5	6.1
Convulsions witnessed on admission	5.1	5.1
Age < 3 years	2.9	6.6

ASTMH POSTER SESSION II

245 RAPID DIAGNOSIS OF MALARIA BY ACRIDINE ORANGE STAINING OF CENTRIFUGED PARASITES. L.S. Rickman, G. Long, R.B. Oberst, J.E. Egan, T.M. Cosgriff, J.D. Chulay, and S.L. Hoffman. Naval Medical Research Institute, Bethesda, MD; U.S. Naval Medical Research Unit No. 2, Manila, Philippines, Walter Reed Army Institute of Research, Washington, D.C.; and U.S. Army Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

A rapid test for the diagnosis of malaria based on acridine orange staining of centrifuged parasites in quantitative buffy coat capillary tubes (QBC<sup>tm</sup>, Becton Dickinson) was evaluated in two studies. In the first, the test was as sensitive as a thick blood smear (sensitivity, 4 parasites/ul) for detecting Plasmodium falciparum parasitemia in 12 volunteers with experimental, sporozoite-induced malaria, and the results were available within 10 minutes of specimen collection as compared to up to 90 minutes for the thick blood smear. The test was next evaluated in a field study in the Philippines. During 4 days, 410 specimens from villagers in an endemic area were studied. When compared to thick blood smear (400 fields by 2 microscopists at 1000x), the test had a sensitivity of 68% and a specificity of 99% in detecting P. falciparum or P. vivax parasitemia. The reasons for the poor sensitivity in the field are unclear, but may have included operator error (a single microscopist read > 100 tests per day), and the effect of the high local temperature and humidity on the parasites during the up to 6 hours between specimen acquisition and processing. The findings indicate that this method may prove to be a valuable adjunct to the clinician who must rapidly diagnose malaria in an acutely ill patient, but may be less useful for large field surveys.

246 PREDICTION OF MALARIA TRANSMISSION POTENTIAL IN CHIAPAS, MEXICO THROUGH USE OF NASA REMOTE-SENSING TECHNOLOGY

D. Strickman, D. Roberts, H. Savage, E. Rejmankova, R. Castro, J. Jimenez, R. Wilkerson, M. Rodriguez and L. Legters. WRBU, Smithsonian, USUHS, Bethesda, MD, UC-Davis, CA and CIP, Tapachula, Mexico

A multidisciplinary project designed to employ remotely-sensed data from NASA satellites to predict the incidence of malaria in the Tapachula area of southern Mexico is in progress. The study area was chosen due to the high prevalence of vivax malaria and the presence of an established research laboratory with expertise in malariology. Initial field work in Mexico consisted of mosquito faunal surveys and qualitative classification of larval habitats during the wet and dry seasons. Taxonomic studies on some 7000 mosquitoes, most with individually associated larval and pupal exuviae, has yielded important information on the composition of the mosquito fauna and the identification of malaria vectors. Studies on habitat characterization has resulted in the association of vector species with specific non-anopheline mosquitoes and habitat types. Work on the seasonal and geographic distribution of productive vector habitats and their association with local land use patterns, the human population, elevation, plant formations, aquatic macrophytes, algae, water chemistry and other factors will produce an initial understanding of malaria transmission in the study area. Future studies will relate the phenology of vector abundance and malaria transmission to remotely sensed data from satellites.

ASTMH POSTER SESSION II

- 247 ADAPTATION AND OPTIMIZATION OF THE FALCON ASSAY SCREENING TEST (FAST-ELISA) FOR TWO-SITE, SINGLE ANTIBODY DETECTION OF PLASMODIUM VIVAX SPOOROZOITES IN INFECTED MOSQUITOES. G.H. Campbell,\* J.D. Sexton, P.M. Procell, and W.E. Collins. Malaria Branch, CDC, Atlanta, GA.

The conventional two-site single antibody ELISAs for detection of sporozoites in malaria-infected mosquitoes are laborious, time consuming, and require complex blocking reagents. FAST-ELISA polystyrene beads on sticks attached to a microtiter plate lid were coated with anti-P. vivax sporozoite monoclonal antibody (NMRI-3). The air-dried capture antibody-coated beads were then exposed sequentially to Anopheles stephensi (ground in 0.4 ml PBS containing 0.5% Tween 20 [PBSTW]), horseradish peroxidase-labeled NMRI-3 in PBSTW, and tetramethylbenzidine substrate for 60, 5, and 5 minutes, respectively. Washing between steps was accomplished by spraying the beads with PBSTW for 20 seconds. After determination of the optimal concentrations and time of incubation of capture and detector antibody, the assay was capable of detecting a minimum of 100 sporozoites per mosquito when 3 standard deviations above the mean of the reactivity of 100 non-infected mosquitoes was used as the cutoff point. Thus, the assay has sensitivity comparable to conventional ELISA methods and the following advantages: no blocking step after capture antibody, dilution of mosquitoes and labeled antibody in only PBSTW, rapid washing conditions, shortened incubation times, and ability to store capture antibody coated beads at room temperature for up to two weeks. After storage of the capture antibody-coated beads, the entire procedure can be accomplished in approximately 1.5 hours compared to 5 hours in the previous assay. Supported in part by USAID PASA BST-0453-P-HC-2086-03.

- 248 SEROEPIDEMIOLOGY OF ANTIBODIES TO THE MAJOR MEROZOITE SURFACE PRECURSOR PROTEIN OF PLASMODIUM FALCIPARUM (GP195) FROM AN ENDEMIC AREA OF THE PHILIPPINES

K. Kramer\*, D. Moffitt, W. Siddiqui and R. Oberst.  
Department of Tropical Medicine, University of Hawaii, Honolulu,  
Hawaii and U.S. Naval Medical Research Unit No. 2, Manila, Philippines.

The importance of gp195 in immunity to P. falciparum (Pf) malaria has been established using animal vaccination experiments. Indications of its role in immunity is also available in humans. Antibodies to gp195 have been detected in humans, however the seroepidemiology of antibodies to gp195 has not been well documented. An epidemiological study was conducted on the island of Palawan to determine the distribution of antibodies to gp195 in a population living in a malaria endemic region. ELISA titers were determined using native gp195 isolated by monoclonal antibody affinity chromatography. The study, conducted on the west coast of the island, was a longitudinal study with samples collected 3 months apart; before and after the transmission season. The slide positive rate for Pf was 8% at the first sample and 19% at the second sample. The overall prevalence rate for anti-gp195 antibodies was 29% (215/748). Paired serum samples were obtained from 249 persons. Fifty-two percent (130/249) remained negative for anti-gp195 antibodies during the study period. Twenty-one percent (53/249) were positive at both sample times, 4% (9/249) converted from negative to positive and 23% converted from positive to negative during the study. The significance of these findings will be discussed. (Supported by USAID)

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249 GAMETOCYTOTICIDAL EFFECT OF THE ANTI-MALARIAL DRUG QINGHAOSU ON  
PLASMODIUM FALCIPARUM.

\_\_\_\_Nirbhay Kumar\*, and Hong Zheng. Immunol. Inf.Dis., School of Hygiene and Public Health, Johns Hopkins Univ, Baltimore, MD.

Qinghaosu, an anti-malarial drug with limited toxicity, has been found to kill not only asexual but also sexual parasites (gametocytes) which transmit the infection from man to mosquitoes. The effect of Qinghaosu in vitro is dependent upon the concentration of the drug as well as upon the initial parasitemia ( $IC_{50} = 1-2 \times 10^{-8}$  M with 1% initial parasitemia). Only the younger stages and not the mature forms of gametocytes were killed by the drug. Resistance of Plasmodium falciparum parasites to other anti-malarial drugs, e.g., chloroquine and pyrimethamine, did not affect susceptibility of asexual and sexual parasites to Qinghaosu. In addition to its proven therapeutic value, Qinghaosu might play a significant role in reducing and interrupting transmission of malaria due to its ability to kill gametocytes.

250 HTLV-I TRANSFORMED T CELL CLONES FROM A PATIENT WITH  
TROPICAL PULMONARY EOSINOPHILIA: IDENTIFICATION OF A TH2  
HUMAN EQUIVALENT

T.B. Nutman\*, C. Steel, G. Delespesse. Laboratory of Parasitic Diseases, NIH, Bethesda, MD and U. of Montreal, Montreal, Canada.

Acute tropical pulmonary eosinophilia (TPE) is an unusual hypersensitivity reaction to filarial infection characterized by wheezing, pulmonary infiltrates, marked peripheral blood eosinophilia and extreme elevations of IgE. In order to understand the mechanisms underlying the elevations of IgE and blood eosinophils and to derive T cell clones capable of specifically regulating these responses, T cells were isolated from a patient with TPE and transformed using the human T cell leukemia/lymphoma virus (HTLV-I). Following infection, these CD3+, CD4+ T cells manifested a fourfold increase in expression of surface interleukin (IL) -2 receptors, a tenfold increase in Fc $\epsilon$  (CD23) receptor expression and acquired viral markers not present in uninfected cells. Cloning soon after viral transformation allowed for the isolation of 21 distinct clones. When tested for the presence of IgE- and eosinophil-regulatory lymphokines, supernatant from one such clone (RV4) was able to: 1) enhance B cell CD23 expression; 2) induce IgE production in vitro; 3) cause eosinophil differentiation in bone marrow cultures; 4) act as an IgA switch factor; and 5) cause resting B cells to be activated and then to proliferate. In contrast, supernatant from this clone had no IL-2 or  $\gamma$ -interferon demonstrable either by functional or immunologic assays. When RNA from this clone was probed with nick translated cDNA cytokine probes (IL-2,  $\gamma$ -interferon, IL-5) in Northern blot analysis, only IL-5 was able to hybridize to RV4. Thus, RV4 appears to be a human analogue of the murine 'TH2' T cell in that it makes IL-4, -5, and -6 and does not make either IL-2 or  $\gamma$ -interferon. Further, the presence of these kinds of T cells in patients with TPE may account for the massive elevations of IgE and eosinophils seen in this disorder.

ASTMH POSTER SESSION II

- 251 DETECTION OF A PHOSPHORYLCHOLINE-CONTAINING PARASITE ANTIGEN  
IN SERA FROM BRUGIA MALAYI INFECTED JIRDS. G.J. Weil.\*  
Washington Univ. School of Medicine, St. Louis, MO.

Sera from Brugia malayi-infected jirds were demonstrated to contain a heat stable, 95-105 kDa parasite antigen by immunoblot with rabbit antibody to the parasite and with a monoclonal AB that binds to phosphorylcholine. This antigen is a major component of B. malayi adult worm excretory/secretory antigen, and it is present in lavage fluid obtained from i.p.-infected animals. The antigen was detected by monoclonal antibody-based enzyme immunoassay (EIA) as early as 4 weeks after infection, and it was present in all sera collected from jirds 9-54 weeks after s.c. injection with 100 or 300 L<sub>3</sub>. Parasite antigen titers were higher in animals infected with the higher L<sub>3</sub> dose (p 0.01).

Antiphosphorylcholine antibodies (anti-PC, measured by EIA inhibition) were present in jird sera for the first 12 weeks after larval injection, but thereafter, antibody titers decreased to undetectable levels. Parasite antigen was not detected by immunoblot or by EIA in sera from 21 human subjects with B. malayi microfilaremia. Antigen may be cleared from human sera by anti-PC antibodies, which were present in all sera tested.

The practical significance of B. malayi antigen detection in the jird is that it provides a sensitive means of noninvasively monitoring the status of infection in this important experimental filariasis model.

- 252 IMMUNOREACTIVITY OF CLONED BRUGIA MALAYI FUSION PROTEINS IN JIRDS,  
MERIONES UNGUICULATUS. J. Yates\*, J. Mooradian, C. Werner, and T.V.  
Rajan, Oakland University, Dept. of Biological Sciences, Rochester,  
MI and Albert Einstein College of Medicine, Bronx, NY.

The current study was undertaken to evaluate the humoral immune response in jirds to cloned B. malayi fusion proteins. Initially serum from a patient with Wuchereria bancrofti infection was used to identify numerous clones in a lambda-gt<sup>10</sup> B. malayi genomic DNA expression library. Recombinant lambda-gt11 clones whose inserts showed strong homology to collagen and myosin DNA sequences attracted our interest because of their strong immunoreactivity with the patient's serum. Groups of age-matched, inbred, male jirds were injected at intervals, with bacterial lysate containing the fusion proteins or with lysate from lambda-gt11-infected bacteria alone, on 3 occasions over a 5 month period. The dynamics of antibody responsiveness during immunization and after challenge infection were evaluated by micro ELISA using a saline-soluble extract of adult female B. malayi. The fusion proteins evoked strong humoral responses during the immunization phase but with no significant anamnestic response after challenge infection. Western blots with the bacterial lysates demonstrated that serum from the immunized jirds, and from jirds with chronic B. malayi infection recognized the fusion proteins. The efficacy of these proteins as potential protective antigens is currently being evaluated.

ASTMH POSTER SESSION II

- 253 EICOSANOID FORMATION FROM ARACHIDONIC ACID BY MICROFILARIAE OF BRUGIA MALAYI: ENHANCEMENT BY HUMAN PLATELETS.  
L.X. Liu,\* P.F. Weller, Beth Israel Hosp, Harvard Medical School, Boston, MA.

We previously reported that microfilariae of B. malayi incorporate arachidonic acid (AA). Since eicosanoid derivatives of AA mediate inflammatory and immunologic actions of mammalian cells, we determined whether this human filarial parasite could also form eicosanoids, which might represent novel mediators of parasite pathogenicity. Because circulating microfilariae interact with platelets, we evaluated whether platelets could modulate microfilarial AA metabolism. B. malayi microfilariae ( $2 \times 10^5$ ) were pre-incubated 30 min with  $^3\text{H-AA}$  ( $10 \mu\text{Ci}$ ), then incubated with ionophore A23187 for 30 min. Lipids were extracted and resolved by HPLC and TLC. No detectable eicosanoids were formed by A23187-stimulated microfilariae alone. However, when human platelets (10-1000 per microfilaria) were incubated with  $^3\text{H-AA}$ -prelabelled microfilariae and A23187,  $^3\text{H-12-hydroxyeicosatetraenoic acid}$  ( $^3\text{H-12-HETE}$ ), a lipoxygenase pathway derivative of AA, was formed in a platelet dose-dependent manner. Incubation of microfilariae with the platelet-derived eicosanoid 12-hydroperoxyeicosatetraenoic acid (12-HPETE), or non-fatty acid peroxides, in the absence of platelets, also resulted in the formation of  $^3\text{H-12-HETE}$ , thereby confirming that the 12-HETE was of microfilarial origin. Thus platelets, by releasing 12-HPETE, promote microfilarial eicosanoid production. 12-HETE, which inhibits *in vitro* platelet aggregation, may enable microfilariae to inhibit platelet aggregation onto their surfaces. These results establish for the first time that a nematode parasite can produce eicosanoids, and suggest that eicosanoids may mediate interactions between parasites and human cells in filarial infections.

- 254 MONOCLONAL ANTIBODIES SPECIFIC FOR BRUGIA PAHANGI TUBULIN.  
N.I. Bughio\*, G.M. Faubert and R.K. Prichard. Institute of Parasitology, McGill University, Montreal, Quebec, Canada

Monoclonal antibodies (Mabs) are powerful tools for studying the immunological and chemical heterogeneity of antigens. Mabs to tubulin can be used to investigate the structure and function of tubulin and microtubules, and to study the interaction of anti-tubulin drugs with tubulin. Mabs have been prepared against both Brugia pahangi and mammalian tubulin, as part of an investigation of differences between nematode and mammalian tubulins and of tubulin as a site for chemotherapy.

Tubulin from B. pahangi was partially purified by Poly-L-lysine affinity chromatography. Pig brain tubulin was isolated by cycles of assembly and disassembly. The tubulin enriched fractions were run on polyacrylamide gels and the tubulin bands cut out. These bands were used to immunize BALB/c mice for the production of anti-tubulin Mabs.

The specificity of anti-B. pahangi tubulin Mabs was assessed by 2-dimensional electrophoresis followed by Western blotting. Two categories of Mabs were obtained: 1) Mabs which react against the tubulins of B. pahangi and Ascaris suum but not against mammalian tubulin; 2) Mabs which react against B. pahangi tubulin but not against Ascaris suum nor mammalian tubulin.

There is a considerable body of evidence that benzimidazole antiparasitic drugs act by binding to parasite tubulin. However, there is little information on the benzimidazole binding site. These Mabs are being used to study the benzimidazole binding site in nematode tubulin. (Research supported by NSERC, FCAR and NIAID)

ASIMH POSTER SESSION II

- 255 THE USE OF CELL-CONDITIONED MEDIUM FOR THE IN VITRO CULTURE OF ONCHOCERCA SPP. LARVAE (NEMATODA: FILARIOIDEA)  
M.S. Cupp\*, E.W. Cupp and C. Pouloupoulou. Dept. of Entomology  
Univ. of Arizona, Tucson, Arizona.

The improvement of in vitro cultivation systems for human filarial parasites is a major requirement to better understand the biochemistry, chemotherapy and immunobiology of this medically-important group of helminths. We examined the effects of co-culture with monkey kidney cells (LLCMK<sub>2</sub>), cell-conditioned medium and decreased atmospheric oxygen on the in vitro molting and viability of infective stage larvae (L<sub>3</sub>s) of Onchocerca lienalis, a bovine parasite, and O. volvulus, the causative agent of "river blindness". O. lienalis L<sub>3</sub>s were cultured in an RPMI 1640-based medium in the presence of an LLCMK<sub>2</sub> cell monolayer or in medium which had been conditioned for three days by cells. Cell-conditioned medium alone in 95% air/ 5% CO<sub>2</sub> produced molting levels of 54 ± 14% which increased to 67 ± 20% in treatments cultured under decreased oxygen; this value equalled the level of molting of worms co-cultured with LLCMK<sub>2</sub> cells. Worm viability in the three environments followed a similar pattern with significant growth and longevity occurring in cell-conditioned medium and decreased oxygen. In seven additional experiments using O. lienalis, overall levels of 74 ± 12% molting and 75 ± 7 % viability on days 21-33 were obtained. O. volvulus molted at an average rate of 77 ± 13% with a mean viability on day 28 of 81 ± 19% (n=4 experiments). Use of cell-conditioned medium and reduced atmospheric oxygen provides a simple method for the long term culture of the mammalian larval stages of Onchocerca species.

- 256 CHARACTERIZATION OF THE THIOL-DEPENDENT DEVELOPMENTAL RESPONSE BY MICROFILARIAE OF ONCHOCERCA LIENALIS AND DIROFILARIA IMMITIS IN VITRO. R.J. Pollack\*, J.B. Lok, and J.J. Donnelly. University of Pennsylvania, Philadelphia, PA.

We previously reported that exogenous reduced glutathione (GSH) enhances development by cultured microfilariae (mf) of O. lienalis in dose-dependent fashion. To elucidate the mode(s) of action of this thiol, analogs of GSH and cyst(e)ine, cysteine delivery agents and inhibitors of enzymes of the δ-glutamyl cycle were tested for effects on growth and motility of microfilariae. Only specific cysteine analogs that possessed a stable sulfhydryl group (relative to cysteine) resulted in development to the late first-stage larva. Inhibitors of δ-glutamyl transpeptidase and δ-glutamylcysteine synthetase failed to affect motility of mf in the absence of GSH or the development of mf in the presence of GSH. No developmental enhancement was observed when GSH was replaced by other reducing agents, radical scavengers, or intracellular GSH precursors. Developmental responses required the continued presence of the cysteinyl-sulfhydryl, a serum component, specific divalent metal ions, and oxygen. Our data further indicate that such thiols function here by specific functional modification of a serum component and as thiol buffers rather than by transport and utilization via the δ-glutamyl cycle, an important consideration in pharmacological studies. Supported by the UNDP/World Bank/WHO and E.M. Clark Foundation.

ASTMH POSTER SESSION II

257 GENETIC CONTROL OF FILARIAL WORM DEVELOPMENT IN DEFINED STRAINS OF AEDES AEGYPTI MOSQUITOES.

A.R. Wattam\*, B.T. Beerntsen, J.M. Aiken, and B.M. Christensen.  
University of Wisconsin, Madison, WI.

Several studies have shown that susceptibility of Aedes aegypti to Brugia malayi can be increased by selection. It has been demonstrated that this susceptibility is controlled by a sex-linked, recessive gene. In order to find the phenotypic expression of this gene, mRNA has been isolated from different tissues in both refractory and susceptible A. aegypti and translated in vitro. Apparent differences between these two populations of A. aegypti have been seen in the mRNA isolated from whole body mosquitoes, thoraces, and Malpighian tubules. Unique peptide differences between the susceptible and refractory strains have been seen in both one and two-dimensional gel electrophoresis. Gel electrophoresis also illustrated differences in the hemolymph from these two strains. A refractory strain has been isolated from the highly susceptible Liverpool strain. Examination of this selected strain could determine if these differences are related to refractoriness and/or susceptibility of A. aegypti to B. malayi. (Supported by NIH grant AI19769.)

258 PHYSIOCHEMICAL DIFFERENCES IN SUSCEPTIBLE AND REFRACTORY STRAINS OF Aedes aegypti (L.) TO INFECTION OF Dirofilaria immitis. J. K. Nayar\*, T. J. Bradley, J. W. Knight and F. Mahmood. Florida Medical Entomology Laboratory, IFAS - Univ. of Florida, Vero Beach, FL and Department of Developmental and Cell Biology, Univ. of California, Irvine, CA.

We have previously reported that the factors which control refractoriness/susceptibility to Dirofilaria immitis infection are present in Malpighian tubule cells of the female Aedes aegypti, Vero Beach strain. However, the gene or genes which control refractoriness/susceptibility also affect other biological parameters of the susceptible and refractory mosquitoes. In investigating these parameters, the physiochemical differences between uninfected and infected susceptible and refractory strains of Ae. aegypti were found to be as follows: a) Bites of susceptible females are less irritating than those of refractory females. b) Life table characteristics showed that the uninfected females of the refractory strain had significantly greater intrinsic rate of increase and higher net rate of reproduction than infected females of the refractory, and both the uninfected and infected females of the susceptible strain. c) Diuresis was directly related to the number of developing or arrested D. immitis larvae in susceptible and refractory infected mosquitoes, respectively. d) Electrophoretic analysis of 15 isozymes of females and their Malpighian tubules of susceptible (SS), refractory (RR) and their hybrid (RS and SR) strains, showed that at least three of the isoenzymes exhibit genetically controlled variations, and e) Differences in morphological characteristics of the Malpighian tubules were observed at both light and electron microscopic levels. The results of these studies support the hypothesis that the genetic selection of Ae. aegypti refractory/susceptible to infection with D. immitis also selects for other biological parameters not directly related to refractory/susceptible condition.

ASTMH POSTER SESSION II

- 259 Competence of certain common Egyptian mosquito species for *Wuchereria bancrofti*. Soliman, B.A.\*, Gad, A.M., Shoukry, A.A., and El Said, S.M. Research and Training Center on Vectors of Diseases, Ain Shams University, Cairo, Egypt.

Five species of Egyptian mosquitoes (*Culex pipiens*, *Aedes caspius*, *Anopheles pharoensis*, *Anopheles multicolor*, and *Anopheles sergentii*) are epidemiologically associated with human filariasis (*Wuchereria (W.) bancrofti*) in several endemic areas; however, there is little quantitative data available regarding the vector competence of these insects. To assess aspects of the vector potential of these species, 5 days old females of each species were experimentally infected by feeding them on human volunteers circulating different microfilaria (mf) counts (5-15, 20-35 and > 35 mf/20 ul blood). All mosquito species acquired the infection with *W. bancrofti* and all but *Ae. caspius* maintained the parasite until the infective stage (L<sub>3</sub>). Infection rates were directly related to the number of mf ingested in all species. Similarly the mean number of L<sub>3</sub> per female was dose dependent except in *Ae. caspius* in which L<sub>3</sub> was detected only in 4 out of 379 females fed 20-25 mf/20 ul blood. Intensity of infections did not significantly affect the survival of *Cx. pipiens*, *An. sergentii*, and *An. pharoensis* females in which >50% survived the extrinsic incubation period of the parasite. The proportion of mosquitoes harbouring L<sub>3</sub> larvae was directly related to the number of mf ingested in all species tested. The highest proportions of such mosquitoes were recorded in case of *An. pharoensis* (76-83%) followed by *An. sergentii* (58-67%) and *Cx. pipiens* (50-56%). However only 20-40% of the infected *An. multicolor* contained L<sub>3</sub>. This is the first experimental evidence that *An. pharoensis* and *An. sergentii* may have the potential to serve as vectors of filariasis. Field observations on feeding habits and survival rates suggests that both species are likely to be candidate vectors of filariasis in nature.

- 260 PRODUCTION AND CHARACTERIZATION OF THREE RAT ANTI-MOUSE EOSINOPHIL MONOCLONAL ANTIBODIES.  
D. Gold\*, W.E. Secor, G.S. Henderson, S.J. Stewart and D.G. Colley. Vanderbilt University School of Medicine and Veterans Administration Medical Center, Nashville, TN.

We have produced 3 rat IgG2a monoclonal antibodies (MAbs) which react specifically with mouse eosinophils in a cellular enzyme-linked immunosorbant assay (cELISA). One of these (6B4), immunoprecipitates a distinct 75 kDa radiolabeled eosinophil membrane component, but fails to react in standard immunoassays, other than the cELISA. By immunofluorescent microscopy (IFAT) and flow cytometry (FACS), the other 2 MAbs (4C6 and 10G2) react strongly with eosinophils. In FACS analysis >85% of eosinophils exhibit high level 4C6 and 10G2 binding. Upon titration, complement-mediated cytotoxicity assays using 4C6 and 10G2 demonstrate differential cytotoxicity for eosinophils. These two MAbs exhibit this activity down to concentrations of 25 ng/ml. The use of these reagents should aid in dissection of the role(s) of eosinophils in a variety of conditions. The effects of these differential, eosinophil-reactive MAbs are now under study in regard to granuloma formation and resistance mechanisms in murine infections with *Schistosoma mansoni*.

(Supported in part by the VA and NIH# AI-11289)

ASTMH POSTER SESSION II

- 261 NATURAL MOLLUSCICIDES FROM EGYPTIAN WILD HERBS  
A.A.I. Elmagdoub,\* M.F. El-Sawy, J.B. Malone, and S.A. Barker.  
College of Agriculture, and High Institute of Public Health, Alexandria  
University, Egypt and School of Veterinary Medicine, Louisiana State  
University, Baton Rouge, LA.

Molluscicidal extracts of Egyptian herbs were studied as potential sources of natural products to replace chemical molluscicides used in schistosomiasis control. Crude water and alcohol extracts of herb 107 were chemically separated to 21 fractions, seven of which retained activity against Lymnaea bulimoides. Acid/neutral and base extraction and dialysis of the aqueous fraction showed that the active compound is a weak acid with high water solubility and a MW of less than 1000. The 'Z' fraction killed 10 of 10 L. bulimoides after 20 minutes at 500 ppm. A crude water extract of herb 558 was 100% effective against Biomphalaria glabrata and L. bulimoides after 24 hr at 500 ppm. A water soluble fraction from a crude acetone extract of herb 558 was 100% effective after 12 hr at 500 ppm, after 48 hr at 250 and 100 ppm, and after 72 hr at 50 ppm. Acute toxicity studies at 50x molluscicidal levels in rats using crude water extracts of herb 107 and herb 558 revealed no mortality or pathologic changes at necropsy 14 days later. Larvae of Aedes aegypti were used as a screen for aquatic arthropod toxicity. A crude water extract of herb 558 inhibited molting and killed 100% of larvae within 7 days at 1000 ppm. The acetone-water extract of herb 558 mixed with a crude water extract of a third herb (LEB) inhibited and killed 100% of larvae after 96 hr at 500 ppm, 120 hr at 200 or 100 ppm, and 144 h at 50 ppm. Patents pending. (Supported in part by the Fulbright Scholar Program).

- 262 SEGREGATION OF BULINUS TRUNCATUS AND HELISOMA DURYI, A COMPETITOR OF  
SCHISTOSOME INTERMEDIATE HOSTS, IN AN EGYPTIAN CANAL HABITAT.  
J.J. Sullivan\*, E. Ruiz-Tiben and M. Habib. Centers for Disease Control,  
Atlanta, GA and Qalyub Center for Field and Applied Research, MOH, Egypt.

Although Helisoma duryi was introduced into the Nile Delta in 1969, this competitor of schistosome snail hosts was first recorded in canals in the vicinity of Kafr Shurafa (Qalyubia Governate) in 1982, 5 years after monthly snail sampling in this village began. The presence of H. duryi in a canal habitat allowed monitoring of this species' behavior in this setting and examination of potential interactions between H. duryi and indigenous Egyptian snails, including B. truncatus.

Monthly sampling of 50 pre-selected sites from April, 1982 to April, 1984 showed highest densities of H. duryi in late spring and early summer with a winter decline. Numbers of B. truncatus from these sites remained low during this period. Biomphalaria alexandrina appeared sporadically.

Samples of all snail species taken at 10 m intervals in one primary canal in March, 1983 and again in March, 1984 suggested a lateral displacement of B. truncatus by H. duryi population. Presence-absence data ( $P < .001$ ) and Sorenson's coefficient (-.487) derived from these samples indicated a negative interaction between H. duryi and B. truncatus, which was not apparent between H. duryi and any of 9 other gastropod species. Although the nature of this negative interaction between H. duryi and B. truncatus is unknown under these field conditions, the segregation of these two species reflects the need for additional field study of the potential of H. duryi in limiting schistosome intermediate hosts in certain transmission foci. [Supported in part by USAID/CDC/MOH Egypt Project SFC (PL-480) 03-327-C].

ASTMH POSTER SESSION II

- 263 A MOLLUSCICIDE ISOLATED FROM THE PLANT TETRAPLEURA TETRAPTERA, O.D. Ngassapa\*, T.O. Henderson, N.R. Farnsworth, C.W.W. Beecher and G.L. Boye, Prog. for Collab. Res. in Pharm. Sciences, Univ. of IL at Chicago, and Dept. of Biol. Chem., Univ. of IL at Chicago, Chicago, IL, and Dept. of Pharmacol., Univ. of Ghana Med. Sch., Accra, Ghana.

Tetrapleura tetraptera (Schum. et Thonn.) Taub. (Leguminosae), a tree which grows in the rain forest zone in West Africa, has been reported to be a potent molluscicide (S.K. Adesina et al., 1980). The methanolic extracts of the ripe fruit and stem bark were found to have LD<sub>50</sub> 3.55 ± 1.25 and 2.0 ppm respectively, on snails of the species Bulinus globosus (S.K. Adesina et al., 1980; Adewunmi & Marquis, 1981b). Our work was initiated in order to isolate and characterize the active constituents from this plant and to determine the minimum concentration at which they are molluscicidal to Biomphalaria glabrata. The ground fruit pericarps and stem bark were extracted, successively, with pet ether, chloroform, methanol and water. The water extract was further fractionated between water and 1-butanol. When tested against Biomphalaria glabrata, the MeOH extract of the stem bark was found to be the most potent (90% lethal at 100 ppm, 24 hr exposure). It was then partitioned between water and CHCl<sub>3</sub>. After concentrating to dryness the water layer gave a 90% mortality rate to the snails at 100 ppm in 24 hrs. It was redissolved in water and further extracted with 1-butanol. The butanol layer, after drying, was 100% lethal at 100 ppm. It was run through a silica gel column and eluted with CHCl<sub>3</sub>-MeOH. This led to the isolation of the molluscicidal compound, as white needles, m.p. 274°. It is active at 1.0 ppm (80% mortality), and is 100% lethal at 5.0 ppm in 24 hrs. It has been characterized as a triterpene glycoside by spectroscopic methods (MS, IR, <sup>1</sup>H-NMR & <sup>13</sup>C-NMR).

- 264 THE COUNTERIMMUNOELECTROPHORESIS (CIEP) FOR THE RAPID DIAGNOSIS OF FASCIOLIASIS AND SCHISTOSOMIASIS IN PATIENTS WITH PROLONGED FEVER AND EOSINOPHILIA.  
E.M. Mikhail\*, Z. Farid, F.G. Youssef, and N.S. Mansour. U.S. Naval Medical Research Unit No. 3, (NAMRU-3).

Ninety-seven acutely ill patients (from rural Lower Egypt where schistosomiasis and fascioliasis coexist) with prolonged fever, eosinophilia, diarrhea and toxemia were examined parasitologically and serologically. Their admission sera were tested by CIEP utilizing purified F. gigantica worm antigen (peaks II and III) and crude and purified (F2 fraction) S. mansoni worm antigen. All sera were first screened by purified Fasciola and crude Schistosoma antigens. Those sera which were positive by both tests were screened with purified Schistosoma antigen to diagnose the mixed infections and to exclude those cross-reacting with the crude Schistosoma antigen. Stools were examined microscopically after MIF Ether concentration once daily for the first week of admission and then once weekly until the beginning of treatment. Serological examination diagnosed 42 fascioliasis cases, 47 schistosomiasis cases and one with mixed infection. Among those serologically positive for fascioliasis, F. hepatica eggs were detected in stools of 23 patients within the first 3 days and of 11 afterwards until the 57th day of admission. The remaining 8 patients were negative until treated. Among those serologically positive for schistosomiasis, S. mansoni eggs were detected in stools of 27 within the first 3 days and of 19 afterwards until the 30th day of admission and one patient remained negative until treated. Seven patients proved to be negative serologically and by repeated stool examination. They were treated for other aetiologic agents. CIEP as described here is a rapid technique for the specific and early diagnosis of fascioliasis and schistosomiasis. (Supported by NMRDC, Bethesda, MD, Work Unit No. 3M162770A870.AQ.320).

ASTMH POSTER SESSION II

- 265 SEROLOGICAL EVIDENCE OF THE APPLICABILITY OF ELISA IN BLOOD SPOTS ON FILTER PAPERS FOR THE CLINICAL STAGING OF HUMAN SCHISTOSOMIASIS MANSONI. K.A. Kamal\*, A. El-Said, H. Hamdto, S. Rashed, and H. Shaheen. U.S. Naval Medical Research Unit #3, Cairo, Egypt, and Benha Medical School, Benha, Egypt.

The relative concentrations of IgM and IgG antibodies (Ab) to Schistosoma mansoni soluble egg antigen (SEA) were evaluated as an indication of the clinical stage of infection. Serum samples and single blood drops on filter papers were obtained from each of 100 school pupils. Enzyme-linked immunosorbent assay (ELISA) was simultaneously performed on diluted sera and on the 24-hour buffer eluates of dry blood spots. The two samples gave similar results in all cases. One group of 15 subjects had IgM:IgG ratios greater than 1 (optical densities (O.D.) in sera and blood spot eluates of  $0.77 \pm 0.32$  and  $0.72 \pm 0.29$ , respectively for IgM and  $0.49 \pm 0.24$  and  $0.50 \pm 0.25$  for IgG). A second group of 10 subjects had IgM:IgG ratios less than 1 (O.D. of  $0.32 \pm 0.07$  and  $0.27 \pm 0.10$  for IgM and  $0.73 \pm 0.36$  and  $0.74 \pm 0.26$  for IgG). Clinical and parasitological findings indicated that the two groups had acute and chronic infections, respectively. Moreover, blastogenic responses of peripheral blood lymphocytes to SEA were higher ( $P = .005$ ) in the acute than in the chronic groups. The study suggests the usefulness of utilizing anti-SEA Abs in blood eluates to differentiate acute from chronic infections, particularly when epidemiologic surveys are intended in endemic areas. (Supported by NMRDC, Bethesda, MD, Work Unit #3M161102BS10.AK.311).

- 266 SEROLOGICAL DIFFERENCE BETWEEN ACUTE AND CHRONIC SCHISTOSOMA MANSONI IN RESPONSE TO KEYHOLE LIMPET HEMOCYANIN (KLH). M.M. Mansour\*, P. Omer-Ali, Z. Farid, A.J.G. Simpson and J.N. Woody. U.S. Naval Medical Research Unit-3, Cairo, Egypt and National Institute for Medical Research, London, England.

The existence of shared epitope between the hemocyanin of the marine mollusc Megathura crenulata, better known as KLH, and an antigen on schistosoma surface has been reported. This antigen was also shown to be a major immunogen in human infection. In the present study the potential use of KLH for the detection of anti-S. mansoni antibodies in sera from 30 patients with acute and 15 patients with chronic schistosomiasis was explored using an enzyme-linked immunosorbent assay (ELISA). Marked differences in IgG and IgM antibody response were noted between acutely and chronically infected patients at a reciprocal serum dilution of 2560. The acute sera had a mean  $\pm$  SD O.D. 490nm values for IgG and IgM of  $1.0 \pm 0.44$  and  $1.34 \pm 0.6$  compared to mean  $\pm$  SD IgG and IgM absorbance for the chronic sera of  $0.22 \pm 0.10$  and  $0.22 \pm 0.11$  respectively. Setting our lowest positive limit at slightly higher than two standard deviations above the mean of the chronic sera, 28 of the 30 patients previously diagnosed as having acute schistosomiasis were correctly identified by their IgG and IgM response. Of 5 patients studied longitudinally IgG levels persisted at the same level 10-13 weeks after treatment. IgM titer, on the other hand, showed a tendency to drop but remained above the established cut off level. This study clearly demonstrates for the first time that quantitative determination of IgG and IgM to S. mansoni schistosome epitopes as measured using KLH and ELISA resulted in a successful simple diagnostic test for differentiating the acute from the chronic forms of the disease. (Supported by NMRDC, Bethesda, MD. Work Unit No.3M161102BS10.AK.311)

ASTMH POSTER SESSION II

- 267 ATTRITION OF ADULT SCHISTOSOMA MANSONI IN A/J MICE.  
M.D. Maloney,\* G.C. Coles, and L.H. Semprevivo. University of  
Massachusetts, Amherst, MA.

In recent years much attention has been focused upon elimination of larval schistosomes whereas little information is available about elimination of adult schistosomes. The purpose of the work described here was to document attrition in Schistosoma mansoni adult populations by identifying dead or dying worms in the mesenteric and portal veins of the A/J mouse, a supposedly permissive host. Mice were infected by tail immersion with S. mansoni cercariae and the adults isolated from the hepatic portal and mesenteric veins at 6, 7, 8, and 10 weeks postinfection. At 8 and 10 weeks, dead worms, predominantly females, were observed among the worm populations. The dead females were irregular in shape, very rigid, and much smaller than the normal adult females. They lacked pigment and were frequently situated within the gynecophoral canals of apparently healthy male worms. Viable eggs were observed within the tissues of one-half of the dead female worms. The cause of death of adult schistosomes in the A/J mouse model is not known, but these data indicate that the phenomenon is fundamentally different from the self-cure phenomena in nonpermissive hosts such as the laboratory rat and may more accurately reflect the human situation.

- 268 IDENTIFICATION OF CHEMICAL STIMULI FOR THE PENETRATION OF  
NECATOR AMERICANUS THIRD STAGE LARVAE IN VITRO.  
Afzal A. Siddiqui\*, Alan C. Fusco and B. Salafsky. Department of  
Biomedical Sciences, University of Illinois College of Medicine, Rockford,  
IL 61107

The stimulation of penetration of Necator americanus third stage larvae was studied in vitro using a gelatin/agar membrane system. It was found that the incorporation of human skin lipids into the gelatin/agar membranes stimulated larval penetration whereas skin proteins and carbohydrates had no effect. Hookworm larvae were not stimulated by TLC purified skin free cholesterol, cholesterol esters, triglycerides and phospholipids, but responded to the free fatty acid fractions of lipids collected from human feet. Essential fatty acids stimulated larval penetration in the following preferential order: Arachidonic acid > linoleic acid > linolenic acid. Various non-essential fatty acids (oleic acid, petroselinic acid, 4,7,10,13,16, 19-decosahexaenoic acid, arachidic acid, palmitoleic acid, myristoleic acid and 11,14-eicosadienoic acid) had very little effect on larval penetration. Studies are in progress to further elucidate other lipids of a stimulatory nature, and whether or not the L<sub>3</sub> larvae utilize these lipids in eicosanoid biosynthesis.

ASTMH POSTER SESSION II

- 269 IN VITRO ASSAY FOR RESUMPTION OF DEVELOPMENT IN INFECTIVE LARVAE (L3) OF SOIL-BORNE, PARASITIC NEMATODES.  
J.M. Hawdon\*, University of Pennsylvania, Phila, PA.

The developmentally arrested free-living L3 (FL3) of hook-worms resume development upon entry into the host, presumably in response to a host-provided signal. To elucidate the signal(s) involved, an in vitro assay for the resumption of feeding in parasitic L3's (PL3) was developed. FL3's are incubated in microtiter wells containing RPMI 1640 (pH 7.2) and 10% serum at 37 C, 5% CO<sub>2</sub>, followed by a 1-2 hr. "feed" with FITC-bovine albumin. L3's ingesting the label are counted under fluorescent light and expressed as a percentage of the total L3's counted. Ancylostoma caninum FL3's begin feeding in 8 h of incubation, and reach maximum activation (70%) at 24 h. The stimulatory factor resides in the >30 kD fraction of ultrafiltrated normal dog serum and is required for activation, since L3's incubated without serum feed at reduced levels (20%). Dog and bovine serum albumin activate FL3's to near maximum in a concentration-dependent manner, suggesting that albumin is the activating molecule, although L3's may begin feeding in the presence of "food" (protein) and permissive conditions. Necator americanus FL3's begin feeding by 48 h, and reach a maximum of 55% by 96 h. in RPMI with 10% normal hamster serum. The difference may reflect the predominant infection route, N. americanus being a skin penetrator, whereas A. caninum infects both orally and percutaneously. The skin penetrator Strongyloides stercoralis behaves like N. americanus. Supported by NIH Grant AI-22662.

- 270 RADIOACTIVE LABELLING OF STRONGYLOIDES STERCORALIS BY FEEDING A SE-75 LABELLED METHIONINE-AUXOTROPHIC ESCHERICHIA COLI TO RHABDITIFORM LARVAE.  
L.M.Aikens\* and G.A.Schad, U. of Pennsylvania, Phila., Pa.

A technique for labelling larvae of Strongyloides stercoralis with selenomethionine-75 is described. An E.coli methionine-auxotroph was grown in Dulbecco's modified Eagles's Medium (DMEM) lacking methionine, but supplemented with 1% cold methionine, 1% lysine, 1% leucine, 5% nutrient broth and 50uCi selenomethionine-75 on a shaker bath for 24 h until the logarithmic phase of growth had ended. Labelled bacteria were washed 3 times by centrifugation, counted in a gamma counter, diluted to a standard concentration, and spread on agarose plates in a monolayer. First-stage larvae were harvested from feces of S. stercoralis-infected dogs. These were washed 6 times in sterile water, and cultured at a concentration of 1000 larvae per bacterially-seeded plate for 7 d in humid air at 30 C. Filariform larvae (L3) were harvested, washed to eliminate free label, and counted in the gamma counter in measured doses. Counts per minute per worm (CPM) were 180 ± 99 with 87 ± 5% of the population labelled sufficiently to produce an autoradiographic focus in a 4-week period. This compared to 11.2 ± 2.1 CPM with 58 ± 4% labelled, when L3s were cultured in nutrient-medium (DMEM with 1% methionine, 1% leucine, 1% lysine, and 50uCi Se-75). Furthermore, the former were capable of penetrating the skin of puppies, whereas the latter had to be injected. Larvae of both groups migrated to the small intestine. Supported by NIH Grant AI-22662.

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Inhibition of the Complement Cascade by Antigen B of Taenia solium.  
Rodriguez, M., \* Torre-Blanco, A., Willms, K. and LaClette, J.P.  
Dept. of Immunology, Instituto de Investigaciones Biomedicas, \* Facultad de Ciencias, UNAM, Mexico 04510, D.F. Mexico.

Antigen B (AgB) is the immunodominant protein in human cysticercosis and has been purified in the form of two polypeptide chains of 95 and 85 kDa. Previous results suggested that AgB is synthesized by the tegumentary cytons of the larva and secreted into the host-parasite interface. AgB has also been found to bind to collagen, allowing its simple and rapid purification by affinity chromatography. AgB also interferes with Complement function in human serum and the present work was carried out to investigate whether inactivation of Complement was due to interaction of AgB with the collagenic moiety of C1q. AgB was purified from a crude extract of T. solium larvae incubated with insoluble collagen fibers from bovine tendon. SDS-Page analysis of eluted material showed that AgB constituted more than 85% of the protein bound to collagen fibers. Normal human serum was preincubated with purified AgB for 60 min at 37 C and C fixation assayed on sensitized sheep red blood cells. Results demonstrated that preincubation of AgB abolished the C activity in the serum. Immunoelectrophoresis of serum incubated with AgB showed that AgB does not induce activation of the alternate pathway, since antibody to factor B did not precipitate a distinct band. ELISA determination using anti-AgB and anti-C1q antibodies demonstrated that AgB binds C1q, suggesting that AgB inhibits the C function through blocking of the C1q active site. This possibility is presently being evaluated.

272 SEROLOGICAL DIAGNOSIS OF CYSTICERCOSIS USING AN ANTIGEN ISOLATED FROM  
Taenia hydatigena CYST FLUID

E. G. Hayunga\*, M. P. Sumner, M. L. Rhoads, K. D. Murrell,  
R. H. Fetterer and R. S. Isenstein. Serology Branch, Pathology and  
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Laboratory, Livestock and Poultry Science Institute, Agricultural Research  
Service; and the Office of the Area Director, Agricultural Research Center,  
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The timely and accurate diagnosis of cysticercosis is a concern for clinicians, veterinary inspectors and public health planners. An ammonium sulfate soluble fraction of Taenia hydatigena cyst fluid, designated ThFAS, was further evaluated for its potential as a diagnostic antigen. Analysis by SDS-PAGE and Western blot revealed a highly reactive, genus-specific, single band with a relative mobility <12kD. Antibodies against this antigen were detected in serum samples from cattle and swine experimentally infected with cysticercosis, but not in Fasciola- or Trichinella- infected animals. IgG and IgM titers were detected as early as 3 weeks post-infection, depending upon the parasite burden. The antigen could be isolated in homogeneous form by HPLC. Recognition of the antigen by human sera with Taenia solium cysticercosis but not Taenia saginata taeniasis infections suggests that the antigen is stage-specific and that it may be of diagnostic value in distinguishing between clinical cases of cysticercosis and taeniasis.

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273 CHARACTERIZATION OF SECRETORY/EXCRETORY PRODUCTS OF SALINE-INCUBATED TOXOCARA CANIS LARVAE.

\*E. J. Villanueva, and W. J. Kozek. Department of Microbiology and Medical Zoology, Medical Sciences Campus, Univ. of Puerto Rico, San Juan, P.R.

Preliminary studies were performed to determine the effect of incubation time on the yield (number and quantity) of secretory/excretory (S/E) components produced by incubated infective larvae of Toxocara canis. Larvae were isolated from embryonated eggs using a Dounce Manual Type Tissue Grinder (7ml), washed and incubated at room temperature in 0.85% sterile saline containing 250 U of penicillin, 250 µg of streptomycin and 0.5 mcg of Fungizone per ml of saline, in batches ranging from 15,000 to 20,000 larvae per ml. Culture medium, collected after 4, 8, 12, 24, 48, 72 hours; 1 and 2 weeks of incubation, was tested for protein content and analyzed by electrophoresis in SDS 10% acrylamide gels. At least ten (10) bands, ranging from 10,000 to 120,000 daltons, were detected in silver stained gels. Some bands were detected as early as 4 hr of incubation; but a pattern of approximately 10 bands observed after 8 hr of incubation was consistently present in samples collected at each succeeding incubation period. Immunodiffusion tests performed with sera from rabbits inoculated with embryonated eggs of T. canis suggested that at least 3 components of larval S/E products are antigenic, but it appears that fewer components are recognized by sera of chronic infections. These studies are being continued to define most efficient method to collect T. canis larval S/E antigens which could be used for serodiagnostic and seroepidemiological applications.

274 PULMONARY IMMUNE RESPONSES TO Nippostrongylus brasiliensis INFECTION IN RATS.

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Using bronchoalveolar lavage (BAL) we have analysed cellular and humoral response in the lower respiratory tract of rats for up to 64 days following primary or secondary infection with 3000 infective larvae of N.brasiliensis.

Compared to total BAL leucocyte number in uninfected animals ( $3.04 \pm 0.3 \times 10^6$ ), the number of BAL cells was significantly increased in infected animals from 4-11 days post infection (pi) in primary (4-5 fold) and from 2-16 days pi in secondary challenges (5-7 fold). Differential counts showed a tremendous increase of neutrophils, alveolar macrophages, eosinophils and lymphocytes on different days pi with anamnestic-like response in secondary challenges.

During the course of infection there was also a significant increase in the total BAL proteins of infected animals. Quantitative analysis of immunoglobulin isotype showed that during 2-4 day pi, there was an increase in total IgG, IgA and IgM in infected animals. By day 8-16 pi, the levels of immunoglobulins were nearly normal. However, by day 21 pi in secondary infection, there was again a significant increase of all immunoglobulin isotypes in BAL. A comparison of albumin to immunoglobulin ratios in both serum and BAL indicated that changes during the early periods following infection were due to serum transudation, whereas during later periods probably involved local immunoglobulin synthesis.

Immunoblot analysis of BAL proteins during the course of an infection showed that parasite-specific antibodies are found in the lower respiratory tract. Moreover, there were qualitative & quantitative differences between serum & BAL parasite-specific antibodies.

Thus, during N.brasiliensis infection there is a pronounced inflammatory & immune response in the lower respiratory tract involving cellular infiltration and vascular permeability followed by local synthesis of parasite-specific antibodies.

This work was supported by Commonwealth Association of Universities, Ottawa and Alberta Heritage Foundation for Medical Research, Canada.

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275 COMPARATIVE ANALYSIS BY FLOW CYTOMETRY OF T CELL SUBSETS IN LUNGS AND SPLEENS OF MICE INFECTED WITH Toxocara canis.

S. G. Kayes, M. B. Stober and R. B. Hester. Department of Anatomy and Cell Biology and Flow Cytometry Laboratory, College of Medicine, University of South Alabama, Mobile, AL.

Bronchoalveolar lavage (BAL) of I. canis-infected mice revealed that from day 8 to 17 postinfection (PI) the BAL contained 80% eosinophils with mononuclear cells and PMN's accounting for the remainder. While spleen cells responded more vigorously to mitogens, BAL mononuclear cells responded more vigorously to I. canis E-S antigens. This study compared the distribution of T cell subsets in the pulmonary infiltrates with the distribution in the spleens of infected mice. CBA/J mice were infected with 250 I. canis ova and BAL cells and spleens obtained 8, 11, 14 and 17 days PI. Cell suspensions from each mouse were stained with monoclonal antibodies against Lyt 1, Lyt 2 and L3T4, and analyzed with the fluorescence-activated cell sorter (FACS IV). Spleen cells from uninfected mice were 32% Lyt 1<sup>+</sup>, 19% L3T4<sup>+</sup> and 12% Lyt 2<sup>+</sup>. BAL was nonproductive using uninfected mice. Following infections, splenic lymphocytes were ~10% Lyt 1<sup>+</sup>, with about 75% of these being L3T4<sup>+</sup>. In contrast, the BAL had 33%, 41%, 30% and 25% Lyt 1<sup>+</sup> cells respectively and almost all BAL T cells were L3T4<sup>+</sup>. Lyt 2<sup>+</sup> cells accounted for about 4% of both populations. Only about 5% of the BAL cells were lymphocytes whereas spleens usually contained 50-75% lymphocytes as determined by light scatter analysis. About 50% of the BAL cells and 90% of the spleen cells within the lymphocyte light scatter gates were not stained by any of the antibodies. That these cells are B lymphocytes is being tested now. Thus, antigen-specific T helper cells which may be producing eosinophil chemotactic factors appear to be accumulating in the lung. (Supported By Grant AI 19968 From NIH)

276 ANTIBODY RESPONSE IN WHITE-FOOTED MICE (PEROMYSCUS LEUCOPUS) EXPERIMENTALLY INOCULATED WITH BORRELIA BURGENDORFERI, THE LYME DISEASE SPIROCHETE.

Tom G. Schwan\*, Karen K. Kime, and John E. Coe. Laboratory of Pathobiology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT 59840.

The white-footed mouse (Peromyscus leucopus) is an important reservoir for the Lyme disease spirochete Borrelia burgdorferi in the northern midwestern and northeastern United States. In the laboratory, these mice remain infected with B. burgdorferi for many months to a year in spite of a pronounced and long-lasting antibody response to infection. To better understand the reservoir competence of white-footed mice, class and subclass specific immunoglobulin responses to different experimental inoculations of B. burgdorferi in these mice were examined by ELISA for 12 weeks (84 days) post inoculation (PI). Mice inoculated with noninfectious (high culture passage) spirochetes produced IgM beginning Day 2 PI and peaking on Day 6 PI. Mice inoculated with infectious (low culture passage) spirochetes also showed an early IgM response, however, there was a secondary greater response during Weeks 2 and 3 PI. Mice inoculated with either infectious or noninfectious spirochetes produced a significant total IgG response beginning Week 2 PI and lasting through Week 12 PI. Specific IgG<sub>1</sub> and IgG<sub>2</sub> responses are presently being examined and will be discussed in relation to their different biological activities relating to persistent spirochetal infections in white-footed mice.

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277 COMPARATIVE ANTIGENIC PROFILES OF EHRlichIA CANIS, E. SENNETSU, E. EQUI, and E. RISTICII.

M. B. Nyindo,<sup>1</sup> I. Kakoma,<sup>2</sup> C. J. Holland,<sup>\*2</sup> R. Hansen,<sup>2</sup> and M. Ristic.<sup>2</sup> International Centre for Insect Physiology and Ecology, Nairobi, Kenya,<sup>1</sup> and University of Illinois, College of Veterinary Medicine, Urbana, IL 61801, USA.<sup>2</sup>

Sera from acute cases of Ehrlichiosis and chronic carriers recognized a 25 kd immunodominant polypeptide conserved among the 4 major Ehrlichia species (E. canis, E. sennetsu, E. equi, and E. risticii). Checkerboard analysis showed that E. canis and E. sennetsu possessed the widest antigenic repertoire, whereas E. equi and E. risticii were the least antigenic. The antigenic hierarchy (complexity) appears to be in the order of: E. risticii → E. equi → E. sennetsu → E. canis based on SDS-PAGE and western blot. These data are discussed in the context of their diagnostic and immunoprophylactic potential.

278 AN IgM-SPECIFIC ANTIBODY-CAPTURE ELISA FOR RIFT VALLEY FEVER DIAGNOSIS IN SHEEP, CATTLE AND GOATS.

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An IgM class capture assay, specific for anti-Rift Valley fever (RVF) virus-antibodies, was developed and applied to sheep experimentally infected with RVF virus. IgM anti-RVF viral antibodies could be detected as early as day 4 postinoculation and attained high levels by day 6. RVF IgM antibodies persisted at high levels through 30 days postinoculation. Data from field populations collected later than 1 year after epizootics suggest that RVF viral IgM antibody is of relatively short duration. The assay employs inactivated RVF antigen and a capture antibody that is reactive with the IgM antibodies of sheep, cattle and goats, thus making it a general rapid diagnostic tool for species of primary importance in the amplification of RVF virus.

Collaborative studies to determine more definitely the duration of IgM class antibodies in these species are currently in progress.

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- 279 Pathogenesis of Cowdria ruminantium infection in goats, as detected through the use of immunohistochemistry.  
C. C. Brown, Plum Island Animal Disease Center, U.S. Department of Agriculture, Agriculture Research Service, Greenport, N.Y. 11944 U.S.A.

Heartwater, an often fatal tickborne disease of ruminants caused by the rickettsia Cowdria ruminantium, is a cause of significant mortality among livestock in sub-Saharan Africa. Pathogenesis of the infection is obscure. Although affected cattle, sheep and goats often die due to severe pulmonary edema and hydrothorax, histologically there is very little inflammation and only rarely can rickettsial colonies be detected morphologically in the lung or myocardium. In contrast, colonies are frequently observed on routine histologic sections in endothelial cells of capillaries in the cerebral cortex. In this study, the presence of C. ruminantium antigen was documented through immunohistochemistry in tissues from animals at various stages of infection. The possible pathogenesis of the infection is discussed.

- 280 Transmission Electron Microscopy of Macrophage Cultures from Cowdria ruminantium-Infected Cattle.  
Sudhir P. Sahu, Foreign Animal Disease Diagnostic Laboratory, APHIS, USDA, P.O. Box 848, Greenport, NY 11944 USA

Macrophage cultures separated from heparinized blood from C. ruminantium-infected cattle were grown in leighton tubes with coverslips and in tissue culture dishes. They were incubated at 37 C for 4 to 5 days in a humid CO incubator. Some of the coverslips were stained with the Giemsa stain and others were used for the direct fluorescent antibody (DFA) test. Cultures in the dishes were used for transmission electron microscopy (TEM).

Various organismal growth forms in vacuoles of the cytoplasm and also outside of the macrophages were seen by the Giemsa stain, DFA test and TEM. Evidence indicates that various forms of inclusion bodies were made from elementary bodies. The inclusion bodies divided and reorganized to form elementary bodies of various shapes and sizes. Elementary bodies were also observed to divide by binary fission.

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DEMONSTRATION AND CHARACTERIZATION OF PROTEASE  
ACTIVITY IN RICKETTSIAL SPECIES OF THE SPOTTED FEVER GROUP

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Galveston, Texas.

Previous data have shown that the expression of rickettsial pathogenicity in vitro and in vivo by rickettsiae of the spotted fever group can be suppressed by amidine-type protease inhibitors. In the present study, we have demonstrated directly the presence of protease activity (PA) in rickettsial suspensions. Sonic extracts of renografin purified Rickettsia rickettsii and R. conorii cultured in yolk sacs contain chromogenic products when incubated with the substrates azocasein and N-benzoyl-DL-arginine- $\beta$ -naphthylamide (BANA). This PA is suppressed by 50mM EDTA and 0.3% SDS and is efficiently extracted by treatment of Triton X-100. Analysis of material from each step of the rickettsial purification from infected yolk sacs using BANA as substrate shows progressive increase of PA per unit of protein and direct correlation between PA and the amount of rickettsiae in the suspensions. On the other hand, significantly lower activity is detected in noninfected yolk sac preparations under the same conditions. Extracts of R. conorii infected L-929 cells show levels of PA significantly higher than noninfected cells. The activity is retained by 0.22 $\mu$ m pore size filters. However, the dissolved PA is a small molecule of 10S. The results of the rickettsial PA characterization in terms of substrate- and inhibitor- specificity, pH optimum and heat inactivation are reported. The PA has been partially purified by affinity and ion exchange chromatography.

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A MOUSE LETHAL DOSE ASSAY FOR DETECTION OF COWDRIA RUMINANTIUM  
(KWANYANGA STRAIN) IN GOATS AND TICKS.

R.G. Endris, T.M. Haslett\*, E.F. Birnie, and W.R. Hess. USDA-ARS,  
Plum Island Animal Disease Center, P.O. Box 848, Greenport, NY.

In the past, detailed quantitative investigations into the capacity of tick populations to acquire, maintain and transmit Cowdria ruminantium, a lethal tick-borne rickettsial pathogen of wild and domestic ruminants, have been hampered by the lack of a simple in vitro assay method and by the lack of a small animal model. With the discovery of isolates that were pathogenic for mice, it became possible to attempt quantitative studies on the tick-Cowdria association. A mouse lethal dose assay was used to detect the mouse pathogenic strain (Kwanyanga) in goats and ticks. The titer of rickettsia organisms in goat blood was directly related to the febrile response of the goat and the rickettsia were undetectable after the fever subsided. The maximum rickettsia titer in goat blood was 10<sup>3</sup> mouse LD50 per ml. Cowdria-infected goat blood was shown to retain infectivity when held on ice for up to 2 hr but when held at room temperature infectivity declined by >50% in 2 hr. The mouse assay detected Cowdria in feeding female Amblyomma variegatum only on the eighth day of feeding and in feeding males on the second and eleventh days of feeding. Cowdria was shown to persist in the hemolymph of the soft tick Ornithodoros coriaceus for a period of at least two years.

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283 EXTRAPOLATION OF A LOUISIANA CLIMATE BASED FORECASTING SYSTEM FOR FASCIOLA HEPATICA TO RAINFALL-DEPENDENT PASTURE ZONES OF FLORIDA AND TEXAS.

J.B. Malone\*, R.A. Rigglesman and R.A. Muller. Louisiana State University, Baton Rouge, LA.

A climate forecasting system has been used since 1984 to advise Louisiana cattlemen each spring and fall of the need for once or twice per year treatment for Fasciola hepatica. An annual index is calculated by accumulating the number of 'growing degree days' (base = 10°C) for days in which moisture is present in the top 2.5 cm of a 15 cm Thornthwaite water budget soil moisture model. For 10 Louisiana climate stations located in flukey areas, annual values are compared to 'normal' values calculated using 30-year average climate data, and low, moderate, high or very high risk year designations are assigned. Annual index values (2 or more years) and 30-year normals were calculated for 3 sites in Florida and 10 sites in Texas. Annual values varied widely at each site, suggesting the value of the forecast, and agreed with historical transmission data. Thirty-year values varied from 418 to 3098 between sites. Laboratory development of F. hepatica eggs through cercariae requires 600 GDD at 25°C. Results suggest 30-year indices provide a typical seasonal transmission profile for a given site and a measure of the severity of the Fasciola problem in divergent climate zones. Temporal patterns indicate that late winter through spring transmission occurs in south Louisiana, east Texas, and north Florida, with less winter development at northern sites. Data further suggest the possibility of 2 transmission seasons (winter and summer) in south Florida and a nearly year-round transmission for the wettest coastal sites of Texas (Angleton) and the lower Mississippi delta (Houma) of Louisiana. Supported by USDA 84CSRS-2-2444.

CUTANEOUS LEISHMANIASIS IN THE GUATEMALAN ARMY.

284 M. Grögl,\* P.W. Kelley, W.R. Ballou, J.D. Berman, D. Gordon, E. Rowton, & R.D. Kreutzer. Walter Reed Army Institute of Research, Washington, DC and Youngstown State University, Youngstown, OH.

During 1987, an epidemiologic and clinical survey of 72 Guatemalan soldiers with presumed cutaneous leishmaniasis (CL) was undertaken. The patients examined were selected with the objective of surveying all cutaneous leishmaniasis patients in a sample of well-defined units that had exposure to the high-risk locations. This method of sample selection estimated that the incidence of CL for troops operating in endemic areas would be at least 2-3% per man-year exposed. Of the 72 patients examined, 43 had needle aspirates and/or 5mm punch biopsies of their lesions cultured on NNN and Schneider's media. Leishmania grew in 21 of 43 cultures (49%). Eleven of the positive cultures were analyzed by cellulose acetate electrophoresis for up to 29 enzyme activities. Eight of the lesion cultures were speciated as Leishmania mexicana mexicana (L.m.m.). Two cultures indicated mixed infections with L. braziliensis braziliensis and L.m.m., and one isolate was identified as L. braziliensis panamensis, a previously unreported species in Guatemala. Twenty-five of 33 (76%) monoclonal antibody touch preparations were positive including 16 of 16 (100%) that came from culture positive patients. Nine of 30 (30%) histopathologic exams (8 of 17 (47%) culture positive) identified amastigotes. Three of 13 (23%) and 8 of 33 (24%) Wright stain and H&E exams were positive. Cutaneous leishmaniasis is a common problem for soldiers in northern Guatemala. The fact that L.b.b. and L.b.p. are now recognized in Guatemala necessitates a reassessment of the currently utilized treatment regimens. Diagnosis by the Leishmania-specific monoclonal IF assay was the most sensitive of the methods used.

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CUTANEOUS LEISHMANIASIS IN HONDURAS CAUSED BY L. donovani chagasi.

Carlos Ponce\*, Elisa Ponce\*, Amy Morrison+, Angel Cruz\* and Franklin Neva#. \*Ministry of Health, Tegucigalpa, Honduras. +U.S. Peace Corps, Honduras. #Laboratory of Parasitic Diseases, NIH

During follow-up and surveillance for visceral leishmaniasis on an island off the Pacific Coast of Honduras, an unusual form of cutaneous leishmaniasis was encountered between January and May 1988. Individuals affected were all children ages 4 to 15 years (5 male and 10 female), lesions were generally few in number (1 to 3), small (1 to 3cm), always papular and non-ulcerative, even when present for several years. Initial clinical impressions of the nature of lesions included sarcoid and leprosy. Cases with cutaneous lesions appeared otherwise well and in good nutritional status. By aspiration of lesions amastigotes were demonstrable in 7 cases tested, and leishmania were cultured from 6 of these. Two of the isolates were typed by Dr. Richard Kreutzer of Youngstown University as L. donovani chagasi by isoenzyme analysis. The same island on which these cutaneous cases occurred was the location where 17 confirmed cases of visceral leishmaniasis were diagnosed between 1977 and 1988. Although there was clustering of all but 2 of the cutaneous cases around houses where previous kala azar occurred, none of the patients with cutaneous lesions were known to have had previous clinical visceral leishmaniasis. Montenegro tests (L. m. mexicana antigen) were done on 14 cutaneous cases; 9 were positive at 5mm or more induration, 1 was borderline and 4 were negative. Several (6) of the cases required two courses of Glucantime injections (15 doses), but all responded to treatment. It is postulated that this unusual form of non-ulcerative cutaneous leishmaniasis represents (1) PKDL in patients who had inapparent kala azar previously, or (2) that the cutaneous cases represent a primary infection with L. d. chagasi with an unusual clinical presentation.

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GEOGRAPHIC DISTRIBUTION AND CLINICAL PRESENTATION OF LEISHMANIASIS IN PERU.

E.D. Franke, C. Lucas, A. Tovar, A. Tejada and F.S. Wignall. U.S. Naval Medical Research Institute Detachment - Lima, Peru and Medicina Tropical - Univ. San Marcos, Lima, Peru.

We have determined the isoenzyme patterns of 102 strains of Leishmania from humans with cutaneous, diffuse cutaneous (DCL) and mucosal disease living in different geographic and ecologic locations in Peru. Cellulose acetate electrophoresis was used to examine the patterns for the following enzymes: MPI, GPI, 6PGDH, ME and LP. WHO reference strains of New World Leishmania were used as controls and for identification of the Peruvian isolates.

ZONE	CUTANEOUS (#)	DCL (#)	MUCOSAL (#)
Jungle	Lbg(15);Lbb(45); Lb?(10);Lma(2)	Lma(1)	Lbb(12);Lb?(4).
Sierra	Lbperuviana?(13)	None	None.

We report the first case of DCL from Peru; the isolate was typed as L. mexicana amazonensis. Isolates from 13 patients from the sierra with cutaneous lesions (uta) had isoenzyme patterns for GPI identical with the braziliensis complex reference strains, but patterns for MPI and ME were distinct from Lbb, Lbg and L. b. panamensis reference strains. There were 14 isolates in the braziliensis complex which showed isoenzyme patterns distinct from those of WHO reference strains for two or more enzymes, although not all 14 isolates had the same isoenzyme patterns. These data indicate that there are several variant enzyme phenotypes of Leishmania in Peru which cause cutaneous and mucosal disease.

ASTMH POSTER SESSION II

- 287 CLINICAL AND EPIDEMIOLOGIC STUDIES OF MUCOCUTANEOUS LEISHMANIASIS (MCL) DUE TO LEISHMANIASIS braziliensis panamensis IN PANAMA.  
\*R.E. Saenz, R. Mata, G. de Rodríguez, A.M. de Vasquez, H. Paz, A. de Pérez, L.A. Quiel. Gorgas Memorial Laboratory, Panama.

MCL in the New World is caused by the Le. braziliensis complex and is the most serious and life-threatening complication of cutaneous (CL). From March 86 to March 88, with the purpose to study the clinical and epidemiological characteristics of MCL caused by Le. b. panamensis, 23 patients with MCL were studied in Santo Tomas Hospital and Gorgas Memorial Laboratory. These patients were 19 to 80 years old, from the endemic rural areas of Panama. 16 were males and 7 females. 14 had a clear history of CL and a characteristic scar, but 9 had no history of leishmaniasis and no scars. Analysis of the previous treatment for the cutaneous infection in the 14 patients, showed that the majority (9) had received no treatment, which appears to be a risk factor for the development of MCL. The interval, between the primary cutaneous lesion and appearance of MCL, ranged from 2 years to 30 years. In 5 patients the mucosal involvement occurred at the time of primary infection, due to the mucosal invasion from a cutaneous lesion in the nose or in the upper lip. The majority (10) had a mild form of MCL, with the infection localized in the nasal mucosa, 9 had moderate infection, 4 of which had perforation of the septum, and only 4 had the severe form of MCL with nasal, oropharyngeal and laryngeal involvement. The most common symptoms on admission were nasal obstruction, rhinorrhea, itching and epistaxis. Patients were treated with intravenous meglumine antimoniate (Glucantime) or sodium stibogluconate (Pentostam) in doses of 20-40 mg of Sb per kg of body weight daily for 20 days. Results of treatment will be reported.

- 288 SCREENING FOR LEISHMANIASIS WITH A TOTAL BLOOD MICRO LYMPHOCYTE PROLIFERATION ASSAY IN A GROUP OF CHILDREN IN JERICHO. S. Frankenburg\*, K. Jaber, R. Alvarado, L. Schnur and C. Enk. The Kuvim Centre for the Study of Infectious and Tropical Diseases, Jerusalem, Israel and the Public Health Department, Jericho.

Cutaneous Leishmaniasis is a parasitic disease characterized by a marked cell mediated response. *In vitro* measurements of this response in humans have so far been used to a limited extent probably because of the large amounts of blood demanded for conventional cell proliferation studies. In many countries endemic for leishmaniasis there are strong cultural and ethical biases against vein puncture under field conditions. The microtest here described enables lymphocyte proliferation to be performed with small amounts of blood (100ul) which can be obtained by finger stick, and do not require separation of mononuclear cells prior to cultivation. The response of individuals immune to L. major was compared to the response after Ficoll separation, and no difference was found between the two methods. The maximal time and optimal conditions for blood storage before testing was determined, and the ability of the assay to evaluate cellular immunity to leishmania was compared to that of the classical Montenegro skin test in a group of thirty school children in the city of Jericho. Defining a positive skin test by induration of more than 5 mm and a positive proliferation assay by stimulation index of more than 2.6 and a response of more than 3000 cpm, we found a significant correlation between the two tests. The proliferation assay was less sensitive than the skin test but somewhat more specific.

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289 Leishmania infantum isolated from dogs in El Agamy, Alexandria - Egypt. M.G. Shehata\*, B.M. El Sawaf, S.M. El Said, S. Doha, J. Dereure, F. Pratlong, and J.A. Rioux. Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt, and Laboratoire d'Ecologie medicale et Pathologie parasitaire, Faculte de Medecine, Universite de Montpellier, France.

Leishmania infantum isolated from 2 out of 80 stray dogs which were captured in October 1987, mainly from open areas were infantile visceral leishmaniasis cases occurred in El Agamy area, Alexandria, a focus of VL in Egypt. The parasite was isolated from the poplyteal lymph node in one dog and from the spleen in another dog. The two isolates were analysed by the iso-enzyme electrophoresis technique and by using 15 enzyme systems and found to be indistinguishable from those isolated from infantile VL cases present in the same area. The prevalence rate of the disease was estimated among the 80 dogs by using the IFA test and found to be 26.4% at  $\geq 1/20$  and 8.3% at  $\geq 1/40$ . The results confirm without any doubt that: 1) the dog is the reservoir host of human VL in the area and 2) the area certainly represents a classical zoonotic focus of VL in the Mediterranean region. Does the El Agamy focus represent the "Last Station" of the true eastward zoonotic VL in the Mediterranean region extending from the western extreme of North Africa, Morocco ?

290 DEVELOPMENT OF LEISHMANIA MAJOR IN PHLEBOTOMUS DUBOSCQI AND SERGENTOMYIA SCHWETZI.

P.G. Lawyer\*, P. Ngumbi, C. Anjili, S. Odongo, Y. Mebrahtu, J. Githure, D.K. Koech, and C.R. Roberts. Kenya Medical Research Institute and U.S. Army Medical Research Unit, Nairobi, Kenya.

The development of Leishmania major was observed in 2 man-biting sand flies, Phlebotomus duboscqi, a known vector of L. major, and Sergentomyia schwetzi, an assumed nonvector. Flies, fed on a leishmanial lesion of a hamster, were dissected at 6-h intervals for the first 48 h postfeeding and at 24-h intervals for the next 8 d. Infection rates were 42% (n=258) in P. duboscqi and 5% (n=162) in S. schwetzi. Transformation from amastigotes to "procyclic" promastigotes occurred in both species between 6 and 18 h postfeeding. In P. duboscqi, the parasites multiplied rapidly and underwent development through as many as 10 forms. Metacyclic promastigotes, the infective form, appeared 6 d postfeeding, first in the region of the stomodeal valve, then in the pharynx, cibarium, and proboscis. In a solitary attempt 14 days postfeeding, a P. duboscqi transmitted L. major to a mouse by bite. In contrast, in S. schwetzi, the parasites multiplied slowly, not developing beyond "procyclic" promastigotes. The parasites did not migrate anteriorly, nor survive beyond 90 h postfeeding, indicating that S. schwetzi is not a potential vector of L. major. Classical strategies for vector incrimination may be confounded by the isolation of non-infective early developmental forms of Leishmania from wild-caught non-vectors.

- 291 TRYPANOSOMA CRUZI ANTIGEN RECOGNITION BY SERA FROM GUATEMALA.  
V.L. Matta\* and J.E. Deas. Louisiana State University Medical  
Center, New Orleans, LA.

A previous epidemiologic survey for Chagas' disease in Guatemala had shown an overall incidence of 8.5%, with that in endemic areas being as high as 20%. Additionally, a positive rate of about 5% was found in blood donors in the capital, a nonendemic area.

The aim of the investigation reported here has been to determine, by various immunological methods, what differences can be found in sera from three groups of individuals. These groups consist of: 1) persons with radiological and clinical symptoms of cardiomegaly, 2) blood donors without any symptomatology and 3) persons who were serologically positive, but without symptoms.

Precipitation of <sup>35</sup>S labeled antigens by the sera from cardiomegalic patients included peptides of approximately 175, 120, 93, 73, 41 and 35 kD; the most heavily precipitated of which was the 93 kD one. The prominence of this band seems to correlate with the state of the disease rather than with serum titer, since ELISA titers of the sera ranged from 1:80 to 1:1,280. In one serum from blood donors, this antigen was also the principal one precipitated. Two sera from the epidemiological study displayed precipitation patterns identical to those given by the sera from cardiomegalic patients. Identical immunoblot patterns using epimastigote antigens and sera displaying the "cardiomegalic" pattern were also seen. These data suggest a possible correlation between recognition of this antigen and state of disease. It also suggests that immunological data may identify individuals at risk for cardiomegaly.

- 292 EXPERIMENTAL CHRONIC CHAGAS' DISEASE IN DOGS. Stephen C. Barr\*, R.A. Holmes, S.P. Schmidt, C. Brown, V.A. Dennis, T.R. Klei. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803

Dogs inoculated subcutaneously with Trypanosoma cruzi (TC) isolates from wild North American hosts (Tc-W) developed acute necrotizing myocarditis which coincided with high parasitemias. Dogs surviving this acute disease entered an indeterminate period during which they were a parasitemic, and clinically normal except for EKG changes. During the 240 day study period, Tc-W infected dogs progressively developed biventricular cardiac dilation and wall thinning, as demonstrated by M-mode echocardiography and angiography. Right sided heart failure preceded left heart failure and death resulted from cardiac insufficiency or severe ventricular arrhythmias within 240 days post-infection (DPI). Dogs infected with TC isolate from a dog (tc-D) remained clinically normal, had low parasitemias and showed no heart changes for as long as 540 DPI. Antibody (Ab) to TC was present in all infected dogs by 25 DPI. Ab levels peaked at 140 DPI and remained elevated after that point. Significant PBMC blastogenic responses to TC antigen occurred at 118 DPI in all infected dogs. These responses peaked at 175 DPI and decreased to preinfection levels by 240 DPI. At necropsy, hearts from Tc-W infected dogs showed severe biventricular dilation, wall thinning and pale myocardial streaking. Hearts from Tc-D infected dogs showed similar but less severe changes. The North American TC isolates used, caused a chronic dilational myocarditis in dogs that is not dissimilar from chronic chagasic myocarditis in man. The cardiac changes and lesions seen are TC isolate dependent. It appears this system would serve as a useful unique model for chronic chagasic cardiomyopathy of man.

ASTMH POSTER SESSION II

- 293 MYOCARDIAL ADENYLATE CYCLASE ACTIVITY IS REVERSED BY VERAPAMIL  
IN ACUTE CHAGAS' DISEASE  
S.A. Morris\*, L.M. Weiss, S.M. Factor, J.P. Bilezikian,  
H.B. Tanowitz, M. Wittner. Albert Einstein College of Medicine, New York, NY.

Chagasic cardiomyopathy caused by *Trypanosoma cruzi* (TC) has many features in common with Syrian hamster cardiomyopathy (SHCM). Since SHCM is modulated by verapamil (VERA), we examined effects of VERA therapy on myocardial adenylate cyclase activity (ACA) in CD1 mice infected with TC. In untreated infected mice isoproterenol dependent ACA measured alone or in the presence of Gpp (NH)p was consistently lower at 21, 21 and 66 days than measurements in uninfected mice (Infected: 72, 75 and 105 pmol/mg/22 min), Uninfected: 135, 115 and 125 pmol/mg/22 min). In contrast, VERA treated infected mice isoproterenol dependent ACA alone or in the presence of Gpp(NH)p at days 21, 31 and 66 was equivalent to the activity present in uninfected but untreated mice. (infected+VERA: 131, 114, 151 pmol/mg/22 min, Uninfected: 132, 105, 120 pmol/mg/22 min). VERA treated uninfected mice isoproterenol dependent ACA measured alone or in the presence of Gpp(NH)p increased two fold (from 135 to 280 pmol/mg/22 min). Further, in the VERA treated infected mice mortality and myocardial pathology were markedly reduced compared to untreated mice. Collectively, these results suggest that VERA ameliorates the clinical, pathological and biochemical characteristics of Chagasic cardiomyopathy.

- 294 VERAPAMIL MODIFIES MURINE CHAGASIC CARDIOMYOPATHY.  
L.M. Weiss\*, S.A. Morris, S.M. Factor, V. Braunstein, H.B. Tanowitz,  
M. Wittner. Albert Einstein College of Medicine, Bronx, NY.

Chagasic cardiomyopathy due to infection with *Trypanosoma cruzi* (TC), has many features in common with Syrian hamster hereditary cardiomyopathy (SHCM). Since SHCM is prevented with oral verapamil (VERA) therapy, we used this treatment in CD1 mice infected with TC Brazil strain. Mice were sacrificed at 17, 33, 45, 60 and 90 days following infection and hearts scored for myocardial fibrosis and inflammation (MFI) in a blinded fashion. Infected mice treated with VERA had significantly less MFI ( $p < .05$ ) at each time point than untreated infected mice, but more MFI than uninfected mice. VERA treatment significantly reduced the mortality rate in infected animals (VERA group: 8%, Control group: 40%;  $p < .01$ ) and decreased the degree of right sided congestive heart failure as manifested by ascites and weight gain. Regardless of therapy with VERA, right heart pathology was greater than left in infected animals. *In vitro* VERA at  $\leq 10$  ug/ml had no effect on TC. We conclude that VERA administration significantly ameliorates the pathological damage and the mortality associated with experimental murine Chagasic cardiomyopathy.

ASTMH POSTER SESSION II

- 295 RAPID, COMPUTER-ASSISTED, IN VITRO, MICRO-METHOD FOR QUANTITATIVE ASSESSMENT OF LEISHMANIA SPP. RESISTANCE TO PENTAVALENT ANTIMONIALS. J.E. Jackson,\* and J.D. Tally. Department of Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC. 20307-5100

Recent reports of treatment failure using pentavalent antimony chemotherapy for human leishmaniases have been made from Italy, India, Africa, South America, and Panama. Unlike bacterial isolates, routinely screened in vitro to confirm drug resistance, most parasite isolates from leishmanial patients are never drug sensitivity tested. The question of whether the parasite is "Sb-resistant" or the patient, "metabolically/immunologically unusual" remains a matter of conjecture. Current in vitro and in vivo drug screening technology relies on a few "well-characterized lab-strains" and is not generally usable or timely for newly isolated Leishmania spp. from all forms of human disease.

We developed a micro-method for parasite drug sensitivity testing and new antileishmanial drug efficacy evaluation. The test is rapid (96 hrs drug exposure + 24 of culture = 120 hrs total); quantitative; conducted in a serum-free, defined medium (eliminating possible drug:serum protein interaction); utilizes the promastigote culture form (making it usable for all leishmanial isolates, not merely those capable of infecting lab animals or cell lines); reproducible; sensitive at drug levels well below those considered achievable in human sera; and readily interpretable (results in computer-assisted, bar graphs). In a retrospective study, including parasites from cutaneous, diffuse cutaneous, mucocutaneous, and visceral disease patients, micro-test results reflected patient treatment outcome.

CRAIG LECTURE

- 296 AEDES AEGYPTI AND AEDES AEGYPTI-BORNE DISEASE CONTROL IN THE 1990S: TOP DOWN OR BOTTOM UP? Duane J. Gubler. Chief, Dengue Branch, and Director of the San Juan Laboratories, Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, San Juan, PUERTO RICO.

SYMPOSIUM: PAUL C. BEAVER - PARASITOLOGY THEN AND NOW

- 297 PAUL BEAVER: AN OVERVIEW OF 60 YEARS OF RESEARCH. R. Jung. Tulane University School of Medicine, New Orleans, LA.
- 298 FILARIASIS THEN. T.C. Orihel. Tulane University School of Medicine, New Orleans, LA.
- 299 FILARIASIS NOW. E.A. Ottesen. Laboratory of Clinical Investigation, National Institutes of Health, Bethesda, MD.
- 300 LARVA MIGRANS THEN. L.R. Ash. UCLA School of Public Health, Los Angeles, CA.
- 301 LARVA MIGRANS NOW. P.M. Schantz. Centers for Disease Control, Atlanta, GA.
- 302 HOOKWORM THEN. M.D. Little. Tulane University School of Medicine, New Orleans, LA.
- 303 HOOKWORM NOW. G.A. Schad. University of Pennsylvania, Philadelphia, PA.
- 304 AMEBIASIS THEN. A. D'Alessandro. Tulane University School of Medicine, New Orleans, LA.
- 305 AMEBIASIS NOW. J.I. Ravdin. University of Virginia, Charlottesville, VA.

DISCUSSION.

SCIENTIFIC SESSION: VIRAL PATHOGENESIS AND VIRAL PERSISTENCE IN NATURE

INTRODUCTION. C.J. Peters. United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

- 306 PATHOGENICITY AND PERSISTENCE OF THOGOTO VIRUS, A CANDIDATE MEMBER OF THE ORTHOMYXOVIRIDAE. P. Nuttall, L.D. Jones, C.R. Davies, T. Booth and O. Staunton. National Environmental Research Council, Institute of Virology, Oxford, UNITED KINGDOM.
- 307 ECOLOGIC FACTORS IN LASSA VIRUS TRANSMISSION. J.B. McCormick. Centers for Disease Control, Atlanta, GA.
- 308 MAINTENANCE AND TRANSMISSION OF HANTAVIRUSES IN RODENT POPULATIONS. J.E. Childs, J.W. LeDuc, G.E. Glass and G.W. Korch. Johns Hopkins School of Hygiene, Baltimore, MD; United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.
- 309 RIFT VALLEY FEVER EPIDEMIC IN MAURITANIA: EPIDEMIOLOGY AND SPECULATIONS ON ORIGINS. J.P. Digoutte. Institute Pasteur, Dakar, SENEGAL.
- 310- EPIDEMIC REPORTS  
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**REVERSAL OF CHLOROQUINE RESISTANCE IN PLASMODIUM FALCIPARUM  
WITH CALCIUM ANTAGONISTS IN THE NANOMOLAR RANGE.**

Alan J. Bitonti, Peter P. McCann and Albert Sjoerdsma  
Merrell Dow Research Institute, Cincinnati, Ohio, U.S.A.

Drug resistance in P. falciparum is increasing in intensity and geographic distribution and is one of the major obstacles to the control of malaria. Combination chemotherapy has been found useful to avoid the problem of drug resistance. It was shown recently that calcium channel blockers such as verapamil and other so-called "calcium antagonists" reverse chloroquine (CQ) resistance in P. falciparum in vitro. MDL 9384 is a calcium antagonist and has also been shown to possess weak antimalarial activity in vitro. Therefore we tested MDL 9384 for possible synergism with CQ. MDL 9384 and CQ proved to be highly synergistic in vitro in both the FCR-3 (CQ-resistant) and clone W-2 (multi-drug-resistant) strains of P. falciparum. MDL 9384 concentrations of 20-500 ng/ml, concentrations which are readily obtained in the plasma of human subjects, were sufficient to completely reverse CQ resistance. Other compounds of this chemical class were also synergistic with CQ. The potent calmodulin antagonist W-7 reversed CQ resistance but was not as effective as MDL 9384. Trials in monkeys are in progress and results of these studies will be discussed. The structures of MDL 9384 and the other compounds in this series will be given in the formal presentation of this abstract.

317 **CHLOROQUINE RESISTANCE IN PLASMODIUM FALCIPARUM AND MULTIDRUG-  
RESISTANT CHINESE HAMSTER OVARY (CHO) CELLS.** B.L. Herwaldt\*, I.Y. Gluzman, D.J. Krogstad, P.H. Schlesinger, V. Ling, S.A.W. Fuqua, A.K. Tandon, and W.L. McGuire. Washington University, St. Louis, MO; Ontario Cancer Institute, Princess Margaret Hospital, Toronto, CANADA; and the University of Texas Health Science Center at San Antonio, TX.

Our recent studies have shown that chloroquine-resistant Plasmodium falciparum accumulates less chloroquine than susceptible P. falciparum because it excretes the drug more rapidly (efflux  $t_{1/2}$  of 2.2 vs >85 minutes). In the studies reported here, we examined the handling of chloroquine by multidrug-resistant (mdr) CHO cells. We also used cDNA probes for the mdr gene and monoclonal antibodies to the P-glycoprotein of mammalian cells to test for reactivity with protein and DNA preparations from susceptible and resistant P. falciparum.

Multidrug-resistant CHO cells accumulated less chloroquine than susceptible CHO cells (10.4 vs 33.7 fmols per  $10^6$  cells). Resistant CHO cells also released chloroquine more rapidly (efflux  $t_{1/2}$  of 5 vs >30 minutes). Furthermore, compounds such as verapamil and vinblastine, which increase the accumulation of chloroquine by resistant P. falciparum, also increased the accumulation of chloroquine by resistant CHO cells. Despite these marked phenotypic parallels, neither cDNA probes for the mdr gene nor monoclonal antibodies directed against P-glycoprotein reacted with material obtained from susceptible or resistant P. falciparum. These results suggest that the genes and proteins associated with drug resistance may vary markedly, even among cells that demonstrate remarkably similar handling of specific drugs, such as chloroquine.

318 REVERSAL OF MEFLOQUINE RESISTANCE IN PLASMODIUM FALCIPARUM IN VITRO.

D.E. Kyle,\* W.K. Milhous, and A.M.J. Oduola. Walter Reed Army Institute of Research, Washington, DC.

Recent observations that chloroquine (CQ) resistance in Plasmodium falciparum can be reversed in vitro represents a novel approach to the study of drug resistance. Since resistance to quinine, quinidine, and desethylchloroquine also can be reversed in vitro, we have examined the ability of calcium antagonists ("broadly defined") to modulate resistance to another quinoline-containing antimalarial, mefloquine (MFQ). Parasites used in this study included a naturally occurring MFQ resistant isolate (Nigeria ST/WRAIR), a clone from a resistant isolate (D-6 from Sierra Leone I/WRAIR), an isolate from a MFQ treatment failure (GH-12, Thailand), a MFQ sensitive and CQ resistant clone (W-2 from Indochina III/CDC), and a MFQ and CQ sensitive clone (HB3). None of the calcium antagonists which reversed CQ resistance (e.g., verapamil, chlorpromazine) were found to reverse resistance to MFQ. Only one calcium antagonist, WR256473, was found to potentiate the action of MFQ versus resistant isolates and clones of P. falciparum without affecting the response of sensitive parasites. The fifty-percent inhibitory concentrations of MFQ resistant isolates and clones were reduced 60-80% by simultaneous exposure with  $2.5 \times 10^{-7}$  M of WR256473. These results suggest that the mechanism of resistance to each of the quinoline-containing antimalarials may be similar in P. falciparum; however, the specificity of the putative receptor involved in the release of drug from resistant parasites may be different for each drug.

319 CYCLOGUANIL AND SULFAMETHOXAZOLE EXHIBIT ENHANCED IN VITRO ACTIVITY OVER SULFADOXINE AND PYRIMETHAMINE.

W.K. Milhous\*, A.M.J. Oduola, D.E. Kyle, P.F. Pierce, L. Gerena, K. Canfield, B.G. Shuster and C.J. Canfield. Walter Reed Army Inst of Res and Georgetown Univ Med Ctr, Washington, D.C & Pharmaceutical Systems Inc, Gaithersburg, MD.

Certain drugs that interfere with folate metabolism, sulfonamides and inhibitors of dihydrofolate reductase, have played an important role in the chemotherapy of and prophylaxis of malaria over the past four decades. Since developing a reliable and reproducible method to assess the intrinsic in vitro activities of antifolate antimalarials (Milhous et al., 1985), we have consistently observed that the active metabolite of proguanil, cycloguanil (CYC), is more potent than pyrimethamine (PYR) against PYR-resistant falciparum malaria and short acting sulfonamides, such as sulfamethoxazole (SMX) are more potent than the long acting sulfonamide, sulfadoxine (SDX). Nanomolar fifty percent inhibitory concentrations of each drug are tabulated below and molar PYR and SDX indices (I) calculated for comparison of activities between the drugs against clones or parasite isolates from various geographic regions:

Parasite Isolate/Clone	CYC	PYR	PYR-I	SMX	SDX	SDX-I
Sierra Leone D-6 (1980)	0.11	0.12	1.09	21.67	108.87	04.83
UNC/Liberia I (1980)	0.62	0.62	1.00	03.62	043.09	11.83
WRAIR/Liberia III (1988)	33.8	188.2	5.16	3815	102016	26.74
Indochina W-2 (1980)	2.06	159.2	77.3	9279	158332	17.06

In combination CYC and SMX also exhibited a marked synergism of drug effect against resistant as well as susceptible parasites. These findings continue to support the prophylactic potential of proguanil in combination with sulfamethoxazole.

O: MALARIA - CHEMOTHERAPY

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PROTECTION AGAINST MALARIA IN MICE BY NUTRITIONAL  
MANIPULATION OF HOST ANTIOXIDANT STATUS.

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Center Tropical Parasitic Diseases, U. Miami, Miami, FL 33177.

Vitamin E (VE) deficiency enhances the therapeutic efficacy in mice of the antimalarial Qinghaosu, which is thought to act in vivo by generation of free oxygen radicals (Federation Proc. 46:1163, 1987). Feeding cod liver oil (CLO) in a VE-deficient diet has a strong antimalarial effect in mice even in the absence of any drug treatment (Exp. Parasitol. 6:555, 1957; FASEB J. 2:A1195, 1988). The purpose of this work was to evaluate nutritional manipulation of host antioxidant status as a possible dietary therapy for malarial infection. Weanling mice were fed a Torula yeast-based diet containing 5% CLO and supplemented with 0 or 100 IU VE/kg. Then the mice were inoculated i.p. with Plasmodium yoelii. Protective effects of the CLO-VE diet after only one week of feeding and over a wide range of inoculum load were seen. Mice fed the CLO-VE diet had normal hematocrits and no sign of hemoglobinuria while mice in the CLO+VE group had depressed hematocrit values. Protective effects on survival and parasitemia were also observed if menhaden oil or linseed oil replaced CLO in the VE-deficient diet. Nutritional manipulation of host antioxidant status appears to be a promising tool for malaria therapy. (Partial support by USDOD contract DAMD 17-85-C-5077).

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IMMUNOLOGICAL CHARACTERIZATION AND MOLECULAR CLONING OF FILARIAL  
ANTIGENS

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and P.J. Lammie. LSU Medical Center, New Orleans, LA; Centers for  
Disease Control, Atlanta, GA; Delta Regional Primate Center,  
Covington, LA; and ICIDR Program, Tulane Univ., New Orleans, LA.

An association exists between the clinical spectrum of filariasis and the *in vitro* immunologic reactivity of lymphocytes from infected individuals to crude parasite extracts. These observations suggest that destructive chronic pathology such as elephantiasis may be a consequence of inappropriate host immune responsiveness to parasite antigens. Purification and characterization of these antigens has been difficult, due to limited quantities of available parasite material; thus, filarial antigens are logical candidates for molecular cloning. A  $\lambda$ gt 11 recombinant cDNA library was prepared from *Brugia pahangi* adult worms. Recombinant clones were identified by immunoblotting with high titered sera from Haitian subjects living in an area endemic for *Wuchereria bancrofti* and by Southern hybridization using DNA probes from *Caenorhabditis elegans*. When lysates of isolated clones were compared to control lysates by PAGE and Western blotting, new bands were apparent. To date, lysates from three of the antibody-selected clones have been compared to control lysates in *in vitro* blastogenesis assays using peripheral blood lymphocytes from filarial antigen sensitized Haitian and U.S. subjects. Two clones (528 and 222) stimulated significant net proliferative reactivity (5000-22,000cpm). Clone 126, in contrast, was not recognized by cells from the same subjects, but was recognized by sera. The other lysates were also tested by ELISA for humoral recognition of the recombinant inserts. DNA sequencing of recombinant clones is currently in progress. Further characterization and immunological analysis of these clones should answer questions about the heterogeneity of human responses to defined filarial antigens. (Supported by AI-16315 and AI-24459).

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SPORONTOCIDAL ACTIVITY OF BRUCEINE A AGAINST A THAI ISOLATE OF PLASMODIUM FALCIPARUM .

K. Pavanand, J. Sattabongkot, N. Lutthiwongsakorn, M. Rasameesoraj, K. Yongvanitchit, and H.K. Webster. USAMC, AFRIMS, Bangkok, 10400, Thailand

We have previously studied the *in vitro* antimalarial activity of Brucea javanica (L.) Merr. against natural isolates of asexual Plasmodium falciparum . One of the active antimalarial compounds isolated, bruceine A, exhibited schizontocidal activity (ID50 = 8.7 ng/ml) against different multidrug resistant isolates of P. falciparum comparable to mefloquine. In herbal medicine records, medicinal plants were mostly prescribed in multidoses. These drugs may therefore also exert sporontocidal activity. We have evaluated the activity of bruceine A against the gametocytes produced *in vitro* from a natural P. falciparum isolate by adding different concentrations of bruceine A to the prepared blood meal immediately prior to mosquito feeding. Anopheles dirus A fed on preparations through membranes were dissected 7-9 days later and the oocysts in the midgut were counted. Bruceine A at 2.75 and 6.0 ng/ml caused a 53 and 76% reduction in the percentage of mosquitoes infected, respectively. Of those mosquitoes infected, there was a 79 and 96% decrease in mean oocyst number. Bruceine A did not affect the health of mosquitoes. These observations suggest a strong sporontocidal activity for the quassinoid, bruceine A.

323 RADICAL CURATIVE PROPERTIES OF WR238605.

G.E. Heisey,\*W.K. Milhous, P. Hansuklarita, A.D. Theoharides, B.G. Schuster and D.E. Davidson, Jr. Division of Experimental Therapeutics, WRAIR, Washington, DC and Armed Forces Res Inst of Medical Sciences, Bangkok, Thailand.

WR238605 (8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-(3-trifluoromethylphenoxy) quinoline succinate) is currently in the final stages of preclinical development at the Walter Reed Army Institute of Research as a replacement drug for primaquine (PQ), a drug which has a poor therapeutic index especially in G6PD deficient patients. Preliminary studies demonstrated that schizonticidal cures of established sporozoite-induced P. cynomolgi infections in rhesus monkeys could be achieved at doses of 0.1, 0.316 and 1.0 mg/kg/day given for seven consecutive days by oral administration in combination with a completely suppressive blood schizonticidal regimen of chloroquine. Calculated CD<sub>50</sub>'s of 0.172 mg/kg/day have demonstrated that WR238605 is 7.4 times as active as PQ (on a molar basis) as a tissue schizonticide in this multiple dose regimen.

The efficacy of single dose regimens of WR238605 was also evaluated in combination with daily doses of chloroquine. Cures were obtained in six of six monkeys at 1.75 mg/kg and one of seven monkeys at 0.875 mg/kg. The calculated CD<sub>50</sub> of 0.742 mg/kg demonstrated that this drug was 6.25 times more effective than the primaquine controls (CD<sub>50</sub> of 4.639 mg/kg) as a radical curative drug administered in a single dose.

The improved efficacy of WR238605, combined with its reduced toxicity, good oral bioavailability and longer half-life make this drug an excellent potential candidate to replace PQ.

O: MALARIA - CHEMOTHERAPY

- 324 MEFLOQUINE PROPHYLAXIS - LACK OF ACCUMULATION ON 250 MG WEEKLY DOSING.  
E.F. Boudreau,\* L. Fleckenstein and L.W. Pang, Walter Reed Army Institute of Research, Washington, DC, and the Armed Forces Research Institute of Medical Science, Bangkok, Thailand

In two malarial prophylactic studies conducted on the Thai-Kampuchean border, multiple dose pharmacokinetics was examined in 45 individuals administered 500 mg mefloquine every 2 weeks and in 38 individuals on 250 mg mefloquine weekly both for 12 weeks. Plasma concentrations of mefloquine were measured by HPLC analysis. Each patient receiving 250 mg weekly had 2 trough plasma drug levels, one at 6-8 weeks and the second at 8-13 weeks into the study. Subjects in the 500 mg study each had a single 6 week plasma level measured. Approximate steady state levels were achieved by six weeks and mean levels (SD) were  $497 \pm 216$  ng/ml on 500 mg every 2 weeks and  $611 \pm 251$  ng/ml on 250 mg weekly. Six week steady state levels were significantly higher ( $p < .03$ , student T test) with the lower weekly dose than with the higher biweekly dose. This is predictable based on the pharmacokinetics of mefloquine elimination. On the 250 mg weekly dosing regimen, mean 12 week levels ( $n = 17$ ) were  $698 \pm 269$  ng/ml. No significant accumulation was seen from 6-12 weeks.

Efficacy of mefloquine 250 mg weekly was 100% and 500 mg every 2 weeks was 92%. No hepatic, renal or hematologic toxicity was found. Mild gastrointestinal symptoms were attributable to the drug on both regimens. Choice of weekly versus every 2 weeks mefloquine prophylactic dosing should consider efficacy, tolerance, compliance and pharmacokinetic factors to maintain a therapeutic non-toxic drug concentration over time.

- 325 MEFLOQUINE THERAPY IN CHILDREN UNDER FIVE IN MALAWI: CORRELATION OF BLOOD DRUG CONCENTRATION WITH PARASITOLOGIC FAILURES. L. Slutsker,\* C.O. Khoromana, D. Payne, L. Patchen, and D.L. Heymann. Malaria Branch and Control Technology Branch, CDC, Atlanta, GA; WHO, Geneva; Ministry of Health, Malawi.

To assess the response of *P. falciparum* to a single oral dose of mefloquine in children <5 years, we studied children attending the outpatient clinic at the district hospital in Mangochi, Malawi. 109 subjects infected with 1,000-150,000 parasites/ul blood (GMPD 20,481/ul) received mefloquine orally, either 25 mg/kg (M25, N=49) or 15 mg/kg (M15, N=60). Capillary blood samples for malaria smears and determination of mefloquine blood concentration were collected on day 0 (D0) and on D2, 7, 14, 21, and 28. Thirty-four *in vitro* microtests (17 per group) showed schizont inhibition at  $\leq 32$  pmol/well. On D7, 6/41 (15%) children in the M25 group were parasitemic, as compared with 2/45 (4%) in the M15 group ( $p > .05$ ). Mean D7 mefloquine concentration was significantly lower in parasitemic children (194 ng/ml) than in aparasitemic children, whether in the M25 (883 ng/ml) or the M15 (600 ng/ml) group. Therapy failure was unrelated to dosage: by D14 32% in the M25 group and 20% in the M15 group had become parasitemic. By D28 this increased to 73% in the M25 and 83% in the M15 group. Analysis of mefloquine pharmacokinetics is in progress to explain this unexpectedly large proportion of parasitologic failures in this age group. Supported by USAID PASA BAF 0421 PHC 22333.

O: MALARIA - CHEMOTHERAPY

- 326 ANTIMALARIAL DRUG USE PRACTICES AND PREFERENCES IN GUATEMALA. T.K. Ruebush II,\* S.C. Weller, R.E. Klein, H. Godoy, and A. Mendez. Medical Entomology Research and Training Unit/Guatemala, CDC, Atlanta, GA; Department of Medicine, University of Pennsylvania, Philadelphia, PA; and Division of Malaria, Ministry of Health; and Universidad del Valle, Guatemala City, Guatemala.

The attitudes and preferences of malaria patients for different antimalarial drugs, routes of administration, and dosage regimens greatly influence their choice of therapy and the effectiveness of that therapy in curing their infections. Surveys conducted during 1987-88 on the Pacific coastal plain of Guatemala showed that 68% of residents prefer parenteral to oral chloroquine (CQ) because of its perceived greater effectiveness, more rapid action and fewer side effects. One ampoule of CQ (150 mg base) is thought to be equivalent to approximately 4 CQ tablets (600 mg base). Although the CQ tablets distributed free-of-charge by the National Malaria Service (NMS) are identical to 2 commercial CQ preparations, they are less popular and are perceived by residents as less effective in curing infections and more likely to cause side effects. Aspirin-containing antipyretic compounds and herbal medicines are recognized as effective in relieving symptoms but not in curing infections. However, 55% of residents believe that such compounds are preferable for the treatment of pregnant women with malaria infections because of the fear of spontaneous abortion with CQ drugs. To increase acceptance and proper use of antimalarial drugs, the NMS will need to correct inappropriate attitudes towards those drugs through community education programs and may need to modify the types of antimalarials they distribute. (Supported by WHO-TDR Project #850077).

- 327 THE RESPONSE OF NIGERIAN CHILDREN WITH PLASMODIUM FALCIPARUM TO CHLOROQUINE AND TO SULFADOXINE/PYRIMETHAMINE. J.G. Breman,\* O.J. Ekanem, L.S. Weisfeld, L.A. Salako, B.L. Nahlen, E.N.U. Ezedinachi, O. Walker, O.J. Laoye, K. Hedberg. Malaria Branch and International Health Program Office, CDC, Atlanta, GA; and Ministry of Health, Nigeria.

To develop treatment policies for a national child survival project, in vivo sensitivity of Plasmodium falciparum to chloroquine (CQ) and sulfadoxine/pyrimethamine (SP) was evaluated in 1987 in children under 5 years of age in 2 areas of southern Nigeria. Responses to 25 mg/kg of CQ or standard doses of SP were assessed. In Oyo State there were no parasitologic failures among 39 children treated with CQ and 42 children treated with SP. Over 91% of these children improved clinically, as assessed by normalization of temperature in children who were febrile (>37.5°C) initially and clinical improvement as judged by parents and clinicians. Improvement in the clinical status was significantly more rapid with CQ than with SP therapy. In Cross River State 62% of 45 children failed to clear parasites after CQ treatment, although the mean parasite density in these children had decreased by over 98%; clinical improvement occurred in 77% of children. SP therapy of CQ-resistant infections resulted in prompt parasite clearance. CQ still remains the recommended first-line therapy in Nigeria for uncomplicated malaria due to its continued clinical efficacy. However, to understand better the changing epidemiology of CQ-sensitivity and the response of P. falciparum to other antimalarial drugs, a nationwide drug sensitivity surveillance network was established. Supported by USAID PASA BAF 0421 PHC 22333.

ASTVM SYMPOSIUM: EHRLICHIOSIS

- 328 THE HISTORICAL BACKGROUND AND GLOBAL IMPORTANCE OF EHRLICHIOSIS.  
Davis Huxsoll, USAMRIID, Fort Detrick, Frederick, MD.

The basic characteristics of the tribe Ehrlichieae include predilection for leukocytic cells where the organisms exist singly or as morulae. The genus Ehrlichia now includes E. sennetsu, a human pathogen. Other ehrlichiae (e.g., E. canis, E. risticii) have been incriminated as potential human pathogens based on serologic evidence in man and experimental infections in non-human primates, respectively. The biggest impact of E. canis was realized during the Vietnam war when thousands of military working dogs died of the disease.

- 329 ADVANCES IN THE IN VITRO CULTIVATION OF EHRLICHIAE. M.B.A. Nyindo and C.J. Holland. University of Illinois, Urbana, IL and ICIPE, Nairobi, KENYA.

Various Ehrlichia species have been adapted to grow in vitro using the method originally developed for propagation of E. canis. The value of this technique in development of diagnostic and immunoprophylactic reagents is discussed.

- 330 BIOLOGIC AND PATHOGENIC PROPERTIES OF EHRLICHIA RISTICII. C.J. Holland. University of Illinois, Urbana, IL.

The isolation, in vitro propagation and characterization of E. risticii, causative agent of Potomac horse fever, was achieved in the last 4 years. The agent has been shown to be antigenically most closely related to the human pathogen, E. sennetsu. The clinical syndrome along with the potential transmissibility by an as yet unconfirmed arthropod vector(s) is discussed.

- 331 ANTIGENIC PROPERTIES OF THE EHRLICHIAE AND OTHER RICKETTSIACEAE. G.A. Dasch, E. Weiss and J.C. Williams, NMRI, Bethesda, MD and USAMRIID, Fort Detrick, Frederick, MD.

Using in vitro propagated organisms, antigenic profiles of selected ehrlichial and rickettsial agents were examined by molecular cloning, chemical characterization of lipopolysaccharide and protein antigens, and by Western immunoblotting. Polyclonal and monoclonal antibodies revealed a major 60 kd polypeptide as a common protein among the organisms investigated.

- 332 BIOLOGICAL PROPERTIES OF EHRLICHIA: SUBSTRATE UTILIZATION AND ENERGY METABOLISM. E. Weiss, G.A. Dasch, J.C. Williams, and Y.H. Kang. NMRI, Bethesda, MD; USMRIID, Fort Detrick, Frederick, MD.

Ehrlichiae harvested from heavily parasitized P388D1 mouse macrophage cells were trypsinized and treated with DNase and separated by either renografin or Percoll density gradient. Glutamate was found to be utilized most rapidly and glucose not at all. In addition, ATP levels were measured. It is concluded that Ehrlichia more closely resemble Rickettsia than Chlamydia.

ASTVM SYMPOSIUM: EHRLICHIOSIS

333 EHRLICHIOSIS IN NONHUMAN PRIMATES. E.H. Stephenson. University of Maryland, College Park, MD.  
Macaca mulatta and M. fasciculatus are susceptible to Ehrlichia sennetsu, a human pathogen. The signs were strikingly analogous to those observed in the human syndrome. Similarly, these animals can be infected with E. equi and E. risticii inducing comparable symptomatology. Additional studies are recommended for E. canis and E. bovis.

334 HUMAN EHRLICHIOSIS. D.B. Fishbein, J. Dawson, M. Mebus, A. Kemp. Centers for Disease Control, Atlanta, GA.  
More than 40 cases of so-called human "spotless" Rocky Mountain fever have been serologically and clinically diagnosed in 11 different states of the USA. Serological evidence and parasitologic observations of intracytoplasmic inclusions and presence of thrombocytopenia is suggestive of an ehrlichial etiology. A history of tick-bite was a consistent feature. Other abnormalities included elevated liver enzymes and pyrexia. It is suggested that Ehrlichia may be an important human pathogen.

335 RECENT RESEARCH FINDINGS ON COWDRIOSIS. J.D. Bezuidenhout. RSA.  
Cowdria ruminantium has been successfully propagated in vitro. This will facilitate antigenic, immunogenic and biologic characterization and eventually lead to vaccine development. Epidemiologically, C. ruminantium has been demonstrated in new tick vectors and in unusual hosts such as guinea-fowls, scrub hare and leopard tortoise. Different strains do not cross protect. Further, C. ruminantium and Ehrlichia are antigenically related.

336 CURRENT STRATEGIES AND PROGRESS IN RESEARCH ON EHRLICHIOSIS. M. Ristic. Keynote speaker, University of Illinois, Urbana, IL.  
The genus Ehrlichia now includes E. canis, E. equi, E. phagocytophila and more recently E. sennetsu and E. risticii. The species typically cause disease in animals but more recently some of the agents have been shown to be potentially zoonotic. The agents causing human infection have not been completely typed. It is suggested that more effort be directed towards antigenic, biochemical and molecular biologic studies pertinent to immunopathology and vaccine development.

337 EVOLUTIONARY HISTORY OF CHLAMYDIAE: ANSWERS FOR SOME OLD QUESTIONS, NO ANSWERS FOR SOME NEW ONES. J.W. Moulder. University of Illinois, Chicago, IL.  
Theoretical and experimental data are being applied to define the phylogeny of Chlamydia, Ehrlichia, Rickettsia and Coxiella, Chlamydia bacteria and Ehrlichia are closely related to Rickettsia whereas Coxiella and Chlamydia are not. It is not known whether Chlamydia is related to Plancomyces and whether host-independent phylogenetic relatives and biovars of the above genera exist.

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- 338 BIOLOGICAL PROPERTIES OF T CELL LINES TO THE MURINE  
MALARIAL PARASITE PLASMODIUM CHABAUDI ADAMI.  
J. Melancon-Kaplan\*, D.M. Russo and W.P. Weidanz, Hahnemann  
University, Philadelphia, PA.

We have demonstrated previously that protection against the murine malarial parasite Plasmodium chabaudi adami (PCA) is mediated by antibody-independent, T cell-mediated immunity. To investigate the mechanism by which protective T lymphocytes are able to resolve infection, T cell lines were generated from the spleen of Balb/c mice sensitized to PCA by immunization with soluble PCA antigen or active parasite infection. The biological activity of the lines was examined. Both types of T cell lines proliferated in an MHC-restricted manner when stimulated with PCA antigen *in vitro*. The T cells appeared to recognize common epitopes shared by PCA, P.c.chabaudi (PCC), P. vinckei petteri (PVP) and Babesia microti (BAB). Cloning of the T cell lines gave rise to T cell clones responding strictly to PCA antigen thereby demonstrating the existence of species-specific determinants. *In vivo*, both types of T cell lines transferred DTH reactivity when injected with PCA antigen in the pinnae of naive Balb/c mice. The DTH response was MHC-restricted and showed cross-reactivity as it was elicited by soluble antigen from PCA, PCC, PVP and BAB. T cell lines from mice recovering from PCA infection were also able to adoptively protect nude mice against acute PCA infection. In contrast, T cell lines from mice immunized with soluble PCA antigen failed to display protective activity suggesting that T cell-mediated immunity to disease and DTH can be dissociated.

- 339 MEMBRANE-ASSOCIATED ANTIGENS OF BLOOD STAGES OF  
PLASMODIUM BRASILIANUM, A QUARTAN MALARIA PARASITE.  
A. H. Cochrane\*, Y. Matsumoto, K. K. Kamboj,  
M. Maracic, R. S. Nussenzweig, and M. Aikawa. Department of  
Medical and Molecular Parasitology, New York University School  
of Medicine, New York, NY and Institute of Pathology, Case  
Western Reserve University, Cleveland, OH.

The localization of Plasmodium brasilianum derived antigens in short and long clefts within the cytoplasm of infected red blood cells, and in association with knobs of the host cell membrane, has been demonstrated by immunogold electron microscopy using monoclonal antibodies produced against blood stages of this parasite. Our results document that malaria-induced short and long clefts, previously distinguishable only by morphology, differ also in their antigenic composition. Another parasite-derived antigen was found to be associated with the parasitophorous vacuole space in schizonts. In segmenters this antigen was present in large amounts between merozoites and in the cytoplasm of infected cells. These antigens have been characterized by biosynthetic labeling and gel electrophoresis.

This work was supported by the Agency for International  
Development (DPE-0453-A-00-5012-00).

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A MONOCLONAL ANTIBODY AGAINST A 50 KD PROTEIN IN MAURER'S CLEFTS OF PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES REACTS WITH HUMAN LEUKOCYTES.

F.W. Klotz,\* A. Szarfman, E. Rock, M. Aikawa, R. Howard, S. Cohen, and L. Miller. Walter Reed Army Institute of Research, Washington, D.C., Naval Medical Research Institute, Uniformed Services University of the Health Sciences, and National Institutes of Health, Bethesda, Maryland, and Case Western Reserve University, Cleveland, Ohio.

Monoclonal Antibodies (mAbs) were obtained by immunizing mice with triton-insoluble extracts from asexual erythrocytic stages of P. falciparum. One of them, mAb FC2, reacts in a diffuse immunofluorescence pattern with trophozoite infected erythrocyte and with a punctate pattern with schizont-infected erythrocytes and mature exoerythrocytic stages. By immunoelectron microscopy, Mab FC2 binds to Maurer's Clefts. On immunoblots it reacts with a triton-insoluble antigen of 50 kD in "knobby" and "knobless" isolates of P. falciparum. Surprisingly, mAb FC2 reacts with a subpopulation of Kupffer cells, 80-90 % of circulating polymorphonuclear leukocytes, and 50 % of circulating monocytes from normal humans not living in malarious areas. The leukocyte antigen has a MW of 12 kD. These data suggest that a P. falciparum protein contains epitopes cross-reactive with a human protein. Some of the autoantibodies in people with malaria may result from such cross-reactive epitopes.

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CHARACTERIZATION OF A PLASMODIUM FALCIPARUM ERYTHROCYTE BINDING ANTIGEN USING AN AFFINITY PURIFIED MONOSPECIFIC ANTIBODY REAGENT.

Palmer A. Orlandi,\* B. Kim-Lee Sim, J. David Haynes, Michael Zegans, and Jeffrey D. Chulay. Department of Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20307.

A 175kD erythrocyte binding antigen (EBA-175) isolated from cultured supernatants of rupturing Plasmodium falciparum schizonts binds to both released merozoites and uninfected erythrocytes (Camus and Hadley. 1985. Science 230:553). The binding of this extracellular protein, an N-acetylneuraminic acid-dependent event, may be important in the initial attachment between the invading merozoite and its erythrocyte host. Antibodies from completely protected infection-immune Aotus monkeys reacted in western blot with small amounts of EBA-175 from both unfractionated culture supernatant and the fraction binding to and eluted from erythrocytes. Affinity-purified monospecific antibody was prepared by elution of antibody from western blots of EBA-175. The monospecific antibody was highly specific for the 175kD protein and did not cross-react with erythrocyte or other malarial parasite antigens. This reagent was used to screen a Camp genomic DNA library in  $\lambda$ gt11 and to isolate a clone expressing a fragment of the EBA-175 gene (see Sim, et al.). Four P. falciparum strains tested with this antibody showed slight molecular weight variations of EBA-175. Studies suggest also that the expression of EBA-175 is stage-dependent, synthesis beginning during late-trophozoite development, and that it is expressed initially as a slightly higher molecular weight Triton X-100 soluble species. The biosynthesis, expression, and binding of EBA-175 to erythrocytes and merozoites may be important events in the invasion of erythrocytes. Thus, EBA-175 may be a suitable antigen candidate in the development of a vaccine cocktail against erythrocyte-stage malaria.

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342 ISOLATION AND CHARACTERIZATION OF THE GENE ENCODING A PLASMODIUM FALCIPARUM ERYTHROCYTE BINDING ANTIGEN.

B. Kim Lee Sim, Palmer A. Orlandi, Gregory S. Leppert, J. David Haynes, Daniel Camus, and Jeffrey D. Chulay. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC. 20307-5100; Department of Immunology and Infectious Diseases, Johns Hopkins School of Public Health, Baltimore; and Unite 42 INSERM, Lille, France.

A 175 kDa Plasmodium falciparum blood stage antigen which binds to erythrocytes and to merozoites, designated EBA-175, appears to be involved in the initial attachment of the merozoite to the erythrocyte, and it has been postulated that antibodies to EBA-175 could inhibit merozoite reinvasion (Camus & Hadley, Science, 1985;230:553). We have characterized and sequenced a 1.8 kilobase EBA-175 gene fragment cloned in  $\lambda$ gt11. The clone was identified by monospecific antibodies selected from malaria immune monkey serum by affinity purification with EBA-175 adsorbed to nitrocellulose. Monospecific antibodies were then selected from malaria immune monkey serum by affinity purification with the expressed protein of the gene fragment and identified authentic EBA-175 in immunoblots. Southern analyses of genomic DNA digested with restriction enzymes Hind III or Xba I and probed with the cloned EBA-175 gene fragment showed no restriction fragment length polymorphism in 5 of 6 geographically distinct isolates of P. falciparum. Based on the deduced amino acid sequence of this EBA-175 gene fragment, several peptides were selected, synthesized, coupled to carrier molecules, and used for immunization studies. This work will enable evaluation of EBA-175 as a vaccine candidate.

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FURTHER CHARACTERIZATION OF PLASMODIUM FALCIPARUM ANTIGENS PRESENT IN IMMUNE CLUSTERS OF MEROZOITES. G. Watt,\* J.A. Lyon and J.D. Chulay. Walter Reed Army Institute of Research, Washington D.C.

When P. falciparum parasites are cultured in the presence of growth inhibitory immune sera, merozoites do not disperse after schizont rupture but instead are agglutinated by antibodies, forming immune clusters of merozoites (ICM). ICM antibodies from immunized Aotus monkeys consistently recognize a restricted number of CAMP strain merozoite surface antigens. The electrophoretic properties of these antigens obtained in two different ways were compared in order to obtain further information on their nature and processing. Schizonts were cultured in growth inhibitory immune sera to give ICM or in medium containing protease inhibitors to give protease-inhibitor stabilized clusters of merozoites (PCM). Antigens from ICM and PCM were separated by 2-dimensional polyacrylamide gel electrophoresis and reacted with antibodies eluted from ICM and with monoclonal and polyclonal antibodies of known specificity. Significant differences between PCM and ICM antigens were found. Antigen gp195 and its processed products p83,p73,p67,p45 and gp45 were recognized in PCM preparations but the processing of p73 to p67 was inhibited in ICM. ICM antibody recognized antigen p101 from ICM but not PCM, but a monoclonal antibody specific for p101 recognized both forms. Possible mechanisms for antibody mediated inhibition of proteolytic processing will be discussed. Regions of surface antigens where proteolytic processing can be inhibited by protective antibody are logical candidates as components of a multivalent vaccine against blood stages of P. falciparum.

- 344 PEPTIDASES FROM SCHIZONTS OF PLASMODIUM FALCIPARUM CULTURED IN VITRO  
 Mark Nwagwu\*, J. David Haynes, Palmer A. Orlandi, Anda Meierovics and  
 Jeffrey D. Chulay. Walter Reed Army Inst. of Research, Washington, DC.

Surface antigens of Plasmodium falciparum are suitable candidates for the development of a malaria vaccine, particularly those antigens which are enriched in immune clusters formed when schizonts rupture in the presence of immune serum. The present study aims to define P. falciparum surface antigens with proteolytic activity. Schizonts of the Camp strain of P. falciparum were lysed by freezing-and-thawing in buffer (150 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 8.0) and a cell extract was obtained by spinning the homogenate at 13,000 g for 2 min. An aliquot of the supernatant was incubated with a variety of fluorogenic peptide substrates in a standard reaction mixture (250 mM NaCl, 100 mM sodium phosphate, pH 7.0) at 25°C for 15 hr. The relative intensity of the released fluorogen, 7-amino-4-trifluoromethylcoumarin (AFC) was determined spectrofluorometrically. The results showed that the cell extract was enzymatically active against MeOSuc-K-L-F-AFC, Suc-L-L-V-Y AFC, MeOSuc-A-A-P-M-AFC, HBr-F-AFC and Glut-G-G-F-AFC but was inactive against MeOSuc-AAPV-AFC and MeOSuc-FPF-AFC. Chymostatin, 1 ug/ml, inhibited by 75% peptidase activity against MeOSuc-K-L-F-AFC. Peptidases active against MeOSuc-K-L-F-AFC, Suc-L-L-V-Y- AFC and HBr-F-AFC have been partially purified on DEAE-cellulose and Sephacryl S-200 columns. A fraction of the peptidase activity against MeOSuc-K-L-F-AFC and Suc-L-L-V-Y-AFC could be specifically adsorbed to monoclonal antibody against a 101kD schizont surface antigen present in immune clusters. This 101kD putative protease may play a role in the release of merozoites or in the processing of merozoite or erythrocyte molecules during invasion.

- 345 IMMUNOLOGICAL STUDIES OF A YEAST RECOMBINANT POLYPEPTIDE BASED ON THE AMINO TERMINAL REGION OF P. FALCIPARUM GP195.  
 S.P. Chang\*, G.S.N. Hui, #P. Barr, #H. Gibson, K. Kramer,  
 A. Kato, and W.A. Siddiqui. Dept. Trop. Med. and Med. Micro., Univ. of Hawaii, Honolulu, HI and #Chiron Corp., Emeryville, CA.

The major merozoite surface protein (gp195) of P. falciparum is under investigation as a potential blood stage malaria vaccine. A 50 kDa polypeptide (195A) corresponding to the amino terminal region of gp195 has been produced in the yeast expression system. The 195A polypeptide was recognized by hyperimmune rabbit antibodies generated against purified gp195 and by sera of monkeys protected from P. falciparum malaria by vaccination with purified gp195. Rabbits immunized with 195A produced antibodies cross-reacting with purified gp195 in an ELISA and bound to blood stage merozoites in an indirect immunofluorescence assay. These results indicate that 195A is immunologically similar to the corresponding region of native gp195. An important aspect of subunit vaccine development is evaluation of the responsiveness to the specific antigen of individuals expressing different immune response genes. A panel of seven congenic mice strains possessing different MHC-linked immune response genes were immunized with purified parasite gp195 and their antibody responses were examined in an ELISA. All seven strains were capable of producing antibodies specific for the complete gp195 protein. In addition, sera from these mice contained antibodies specific for the 195A polypeptide. These results suggest that individuals expressing many different immune response gene are capable of producing antibodies recognizing this region of the complete protein. The significance of this region of the gp195 molecule in the development of protective immunity is currently under investigation. (Supported by USAID.)

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- 346 LACK OF CORRELATION BETWEEN IMMUNITY TO MALARIA AND IN VITRO PRODUCTION OF GAMMA-INTERFERON BY T LYMPHOCYTES CULTURED IN THE PRESENCE OF PLASMODIUM FALCIPARUM ANTIGEN. C. Chizzolini,\* G. Grau, A. Geino, and D. Schrijvers. Centre International de Recherche Medicale, Franceville, Gabon; and WHO International Research and Training Center, University of Geneva, Geneva, Switzerland.

T cell-mediated immunity is reported to play a key role in defense against malaria, possibly through the production of interferon (INF). INF-alpha and INF-gamma were measured by radio-immunoassay in supernatants from cultures of peripheral blood mononuclear cells (PBMC) or purified T cell subsets incubated with either Plasmodium falciparum schizont-enriched antigen (PFAG), uninfected red blood cells (RBC) or pokeweed mitogen (PWM). Results with PBMC from 7 Europeans with a history of a single malaria infection during the previous 1-9 months, and from 24 healthy African adults living in a P. falciparum endemic area (Haut-Ogooue, Gabon), were as follows: a) INF-gamma was produced only by PBMC cultured with PFAG or PWM. b) PFAG-induced production of INF-gamma was dose-dependent and due to CD4+ T cells; CD8+ T cells had no regulatory activity on INF-gamma production. c) PFAG-induced cell proliferation was significantly associated with INF-gamma production ( $r=0.83$ ,  $p<.001$ ); significantly higher levels were detected with European PBMC ( $34.92 \pm 7.02$  U/ml) than with African PBMC ( $4.89 \pm 2.70$  U/ml) ( $t= 4.89$ ,  $p<.001$ ); no such difference was found with PBMC cultured with PWM. d) No INF-alpha was detectable. Thus, malaria parasite antigens induce the *in vitro* production of INF-gamma by CD4+ T cells, but individuals considered to be clinically resistant to malaria are non- or low producers of INF-gamma.

- 347 INCREASED LEVELS OF SOLUBLE INTERLEUKIN 2 RECEPTORS IN PLASMODIUM FALCIPARUM MALARIA. P. Nguyen-Dinh\* and A.E. Greenberg. Malaria Branch, CDC, Atlanta, GA.

Soluble interleukin 2 receptors (IL2-R) are released *in vitro* by activated human peripheral blood mononuclear cells and are increased in several clinical situations. Levels of IL2-R were determined with a capture two-site immunoassay in sera from patients with P. falciparum malaria in Kinshasa (Zaire) and from age-matched healthy controls both in Kinshasa and the United States. Levels of soluble IL2-R in 41 children with clinical malaria were significantly higher than in 33 healthy children (geometric means: 4405 and 1037 U/ml, respectively). Children who had malaria with cerebral complications had significantly higher serum IL2-R levels than those without. Among healthy children, no difference in soluble IL2-R was found between those asymptotically infected with P. falciparum and those who were not. However, healthy children in Kinshasa had significantly higher soluble IL2-R levels than healthy U.S. children. Adults in Kinshasa with symptomatic malaria had levels of serum IL2-R (1376 U/ml,  $n=22$ ) higher than healthy adults either in Kinshasa (296 U/ml,  $n=20$ ) or in the U.S. (356 U/ml,  $n=16$ ). In a subset of the Kinshasa subjects, an elevation in soluble CD8 antigen was found in the serum of malaria patients, while this was not the case for IL2 or gamma-interferon. The IL2-R findings may indicate activation of T-cells (including suppressor/cytotoxic cells) during P. falciparum malaria. Studies on soluble cell markers and cytokines can provide useful information for understanding immunoregulation in P. falciparum malaria. Supported by USAID PASA BST-0453-P-HC-2086-03.

348 CHANGES IN PLASMA TUMOR NECROSIS FACTOR ALPHA (TNF) AND INTERLEUKIN-1 BETA (IL-1) FOLLOWING CHALLENGE OF NONIMMUNE OR IMMUNE HUMAN VOLUNTEERS WITH PLASMODIUM VIVAX. C. Muñoz, J.R. Murphy, S. Baqar, J. Davis, D.A. Herrington, and M.M. Levine. Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland 21201.

To determine the relationship between levels of these cytokines and course of P. vivax infections, blood was collected before and at intervals after infected mosquitoes had fed on 2 volunteers. One had no history of malaria, the other had had two infections with P. vivax. The naive volunteer developed patent infection 11 days after mosquito bites, and peak parasitemia (334,400 parasitized erythrocytes/ml) and peak fever (40.9 C) on day 14 (therapy was then initiated); TNF and IL-1 were depressed to below prepatent levels during parasitemia. The volunteer with previous contact with the parasite became patent on day 11 and had peak parasitemia (37,800,000/ml) and peak fever (40.1 C) on days 19 and 18, respectively, TNF was markedly elevated during high parasitemia, IL-1 was sporadically elevated. The plasma cytokine responses differed as a function of prior exposure to parasites but did not correlate with resistance to infection.

349 Immunological Studies of the Effects of Plasmodium falciparum Hemozoin on Normal Human Mononuclear Cells. P.Goldie\*, F.T.Valentine, and J.P.Vanderberg  
Department of Medical and Molecular Parasitology, Department of Medicine, Infectious Diseases and Immunology Division  
New York University Medical Center, NY, NY.

Plasmodium falciparum hemozoin, purified from in vitro blood stage culture and biochemically characterized, was studied to determine its effects on normal human mononuclear cells. Studies on cytotoxicity, proliferative response, antigen presentation, phagocytosis, cytokine production and surface activation marker expression were performed. Comparisons of various effects of hemozoin were made with hematin, the major non-protein component of hemozoin. Hemozoin was found to be non-cytotoxic to mononuclear cells and is a non-specific mitogen. Normal primed responses to tetanus toxoid were suppressed by preincubation of antigen presenting cells with hemozoin. Hemozoin ingestion by monocyte-derived macrophages enhanced subsequent phagocytosis of opsonized sheep erythrocytes and latex beads. Cytokine release and surface activation expression of macrophages were also affected and will be discussed. These results suggest ingestion of hemozoin by hepatosplenic macrophages can explain certain immunologic alterations observed in malaria.

Graduate research supported by NIH National Research Service Award 5T32 AI-07180.

Q: ARBOVIROLOGY - MOLECULAR BIOLOGY AND VACCINE DEVELOPMENT

350 SYNTHETIC PEPTIDES DERIVED FROM THE DEDUCED AMINO ACID SEQUENCE OF THE E-GLYCOPROTEIN OF MURRAY VALLEY ENCEPHALITIS VIRUS ELICIT ANTI-VIRAL IMMUNITY.

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We have used computer analysis to study homology, hydrophilicity, and secondary structure, of the deduced amino acid sequence of the envelope (E)-glycoprotein of the flavivirus, Murray Valley encephalitis (MVE). Using these predictions, we have modified the proposed E-glycoprotein antigenic structure of Nowak and Wengler. In our model, the R1 encompasses the disulfide stabilized amino-terminal region, as well as some sequences of the R3 which are brought into R1 proximity by predicted folding. Using this information we have prepared eleven synthetic peptides and analyzed their immunogenicity. Peptides derived from the R1(R3), and R2 domains are capable of eliciting anti-viral antibody. All of these peptides are recognized by polyclonal antiviral antibodies, with peptides derived from the R1(R3) domain demonstrating MVE virus specificity. One peptide from the R1 elicited low level virus neutralizing antibody. Using either competitive binding assays with anti-peptide antisera and radioactive monoclonal antibodies (MAbs), or direct binding assays with peptides and MAbs, a number of previously identified epitopes could be localized.

351 ANTIGENICITY AND IMMUNOGENICITY OF RIFT VALLEY FEVER VIRUS GLYCOPROTEINS EXPRESSED BY BACULOVIRUS RECOMBINANTS.

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The Rift Valley fever virus (RVFV) M segment, which encodes the viral glycoproteins (G1 and G2), was inserted by homologous recombination into the polyhedrin gene of the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV). In *Spodoptera frugiperda* (SF-9) cells, recombinant viruses directed synthesis of RVFV G1 and G2 that had antigenic properties and gel electrophoresis mobilities similar to their authentic counterparts derived from RVFV-infected Vero cells. SF-9 cells infected with recombinant AcNPV, and thus synthesizing RVFV glycoproteins, were shown to be excellent sources of antigenic material for conventional diagnostic immunoassays (e.g., ELISA). To test possible vaccine relevance, mice were immunized with crude homogenates of SF-9 cells that contained RVFV G1 and G2 expressed by recombinant AcNPV. Such mice, in contrast to those injected with normal SF-9 cells, responded by: 1) producing antibodies to RVFV G1 and G2 (but not other RVFV proteins) as measured by ELISA, immunoprecipitation, and neutralization of viral infectivity in vitro, and 2) surviving challenge with more than 1000 LD<sub>50</sub> of RVFV.

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352 USE OF DENGUE VIRUS STRUCTURAL PROTEINS AND NONSTRUCTURAL PROTEIN NS1 PRODUCED BY RECOMBINANT BACULOVIRUS FOR IMMUNIZATION AGAINST DENGUE VIRUS INFECTION.

Y.M. Zhang, D.R. Dubois, K.H. Eckels, R.M. Chanock and C.J. Lai\*.  
Laboratory of Infectious Diseases, NIAID, NIH, Bethesda, MD and Walter Reed Army Institute of Research, Washington, D.C.

Dengue virus infections continue to cause major epidemics in many geographic areas. The use of genetically engineered dengue proteins for immunization represents a promising vaccine strategy. We have employed the high yielding baculovirus-insect cell system to achieve expression of dengue viral proteins from cloned DNA sequences. A recombinant baculovirus containing dengue cDNA that codes for the three structural proteins, i.e., capsid (C) protein, membrane (M) protein, and envelope (E) glycoprotein and nonstructural proteins NS1 and NS2a was constructed. Infection of cultured insect cells with this recombinant virus produced what appeared to be authentic E and NS1. Immunization of mice with the dengue protein products induced resistance to fatal dengue encephalitis. To further elucidate the protective immunity, recombinant baculovirus that expresses only E or NS1 was constructed. Mice immunized with the infected cell lysate containing either E or NS1 developed complete resistance to dengue virus challenge. The immunogenicity of these dengue viral proteins will be presented.

353 RECOMBINANT VACCINIA VIRUSES EXPRESSING DENGUE 4 STRUCTURAL OR NON-STRUCTURAL PROTEINS PROTECT MICE AGAINST LETHAL CHALLENGE.

M. Bray,\* B.T. Zhao, B. Falgout, K.H. Eckels and C.J. Lai.  
Laboratory of Infectious Diseases, NIAID, NIH, Bethesda, MD and Walter Reed Army Institute of Research, Washington, D.C.

The lack of effective vaccines against the dengue viruses has prompted interest in the use of vaccinia virus as a vector for expression of protective dengue antigens. We have constructed a number of recombinant vaccinia viruses expressing either multiple or individual dengue glycoproteins. In a series of experiments, 22 out of 23 mice immunized with a control vaccinia recombinant died following intracerebral challenge with 100 LD50 units of mouse-adapted H241 dengue 4. Immunization with a recombinant expressing authentic structural pre-membrane (pM) and envelope (E) glycoproteins and the nonstructural protein NS1 resulted in survival of 26 out of 27 animals following challenge. All 14 mice vaccinated with a recombinant expressing only the structural proteins were completely protected. A recombinant expressing apparently authentic NS1 alone also produced 100% survival in 15 mice. The ability of these recombinants to protect against cross-challenge by type 1 and type 2 dengue virus is under investigation.

Q: ARBOVIROLOGY - MOLECULAR BIOLOGY AND VACCINE DEVELOPMENT

- 354 EXPRESSION OF THE M AND THE S GENOME SEGMENTS OF HANTAAAN VIRUS BY VACCINIA AND BACULOVIRUS RECOMBINANTS.  
C.S. Schmaljohn, A.L. Schmaljohn, and J.M. Dalrymple. United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

Hantaan virus, the etiologic agent of Korean hemorrhagic fever, is the prototype member of the Hantavirus genus of Bunyaviridae. Like other viruses in the family, Hantaan has a tripartite, single-stranded RNA genome, and utilizes its small (S) and medium (M) genome segments to encode the viral nucleocapsid (N) and envelope (G1 and G2) proteins, respectively. Two eucaryotic viral vectors, vaccinia virus and Autographa californica nuclear polyhedrosis virus (AcNPV), were used to express Hantaan N, G1 and G2. Noncoding regions of the 3' and 5' ends of Hantaan M and S cDNAs were modified by site-directed mutagenesis or limited exonucleolytic digestion to remove homopolymeric tails and to generate appropriate restriction sites for transfer vector construction. Restriction fragments containing coding information for only G1 or G2 were also prepared. Modified cDNAs were subcloned into the transfer vectors pAcYM1 or pSC-11 and homologous recombinations with AcNPV or the Connaught or WR strains of vaccinia virus were performed. Hantaan virus G1, G2, N or G1 and G2 were expressed by both the AcNPV and vaccinia virus systems. The G1 and G2 envelope glycoproteins, which are encoded in a single long open reading frame in Hantaan M, appeared to be cleaved and glycosylated in both systems. All of the expressed proteins were antigenic in classical serologic tests and immunogenic in research animals. The potential use of the expressed products as diagnostic reagents or candidate vaccines is being explored.

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PROTECTION AGAINST DENGUE AFFORDED BY IMMUNIZATION WITH YELLOW FEVER 17D

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Cynomolgus macaques without prior flavivirus exposure were immunized with yellow fever 17D vaccine; all developed yellow fever neutralizing antibodies at titers  $\geq 1:80$ . Sixty-eight days later, 4 yellow fever immunes and 4 control monkeys were challenged with  $10^5$  mosquito cell ID<sub>50</sub> of dengue type 2 strain 16681. None of the animals developed clinical signs, fever or leukopenia. Viremias assayed by intrathoracic inoculation of Toxorhynchites were significantly shorter (mean 1.0 day) and peak titers lower (mean 3.7 ID<sub>50</sub>/ml) than in controls ( $>5.0$  days, 5.0 ID<sub>50</sub>/ml, respectively). These results suggest that yellow fever immunization might be employed as a means of preventing dengue transmission in a population. In addition, the data may provide an explanation for the absence of dengue epidemics in areas of high yellow fever endemicity.

Q: ARBOVIROLOGY - MOLECULAR BIOLOGY AND VACCINE DEVELOPMENT

356 IMMUNIZATION WITH LIVE-ATTENUATED DENGUE TYPE 4 (341750 CARIB)  
VIRUS VACCINE.

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A live-attenuated vaccine was developed from dengue 4 (341750 CARIB) (one mosquito, 20 primary dog kidney cell and 4 fetal rhesus lung cell passages). The virus exhibited decreased infectivity for primates:  $2.4 \times 10^5$  mosquito infectious doses of the vaccine virus, as compared to 60 of the parent, were required to infect rhesus monkeys, and was selected for human testing.

To determine safety and immunogenicity, a single 1 cc dose of the vaccine was given subcutaneously to three pairs of flavivirus non-immune volunteers, step wise in increasing concentrations. At the 1/100 dilution, one of two became viremic between days 12 and 15, experienced a mild elevation of temperature (99.4°F) and developed HAI (Titer=1:40), neutralizing (1:30) and ELISA IgG and IgM antibodies. Neither recipient of the 1/10 dilution became infected. One recipient of the undiluted vaccine had negligible symptoms and developed short-lived HAI (1:10) antibody. Activation of T cells was observed in the two recipients of the undiluted vaccine. We conclude that the DEN 4 CARIB vaccine is over attenuated and minimally immunogenic for man at this passage level. Lower passage levels that are being developed may be more appropriate as future vaccine candidates.

357 EVALUATION OF A LIVE, ATTENUATED, VENEZUELAN EQUINE ENCEPHALOMYELITIS  
VACCINE (TC-83)

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Venezuelan equine encephalomyelitis (VEE), a mosquito-borne, viral disease endemic to many geographic areas, can cause significant human disease of epidemic proportions. A live, attenuated VEE Vaccine (TC-83) suitable for use in man was developed and first tested in human subjects in 1962. This vaccine is used at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) for primary vaccination of individuals identified to be at potential risk of exposure to virulent VEE virus. From March 1975 to September 1987, 767 individuals were vaccinated with TC-83. For the purpose of this study, data are reported on the 679 individuals with negative, pre-vaccination, plaque-reduction neutralization titers (PRNT<sub>80</sub>). We saw no significant statistical difference in the serological response on the basis of sex or age. Post-vaccination titers obtained prior to day 14 were lower than titers obtained between 3-6 weeks after vaccination. Initial post-vaccination titers revealed that 20% of vaccinees did not obtain PRN<sub>80</sub> titers of 1:20, presently considered to be a protective level. Local reactions at the injection site were seen in six vaccinees (6/679). Systemic reactions were noted in 123 vaccinees (123/679). The reactions were evaluated on the basis of severity of signs and symptoms and their occurrences after vaccinations. The systemic reactions were distributed in a biphasic pattern with 32.8% occurring the first 3 days after vaccination and the remainder from 4 to 24 days post vaccination.

Q: ARBOVIROLOGY - MOLECULAR BIOLOGY AND VACCINE DEVELOPMENT

358 FURTHER STUDIES ON THE SAFETY AND PROTECTIVITY OF A MUTAGENIZED RIFT VALLEY FEVER VIRUS IMMUNOGEN.

J.C. Morrill,\* L. Carpenter, D. Taylor, H. Ramsburg, J. Dalrymple, H.W. Lupton, and C.J. Peters. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701.

A mutagen-attenuated Rift Valley fever virus (RVFV), MV P12, was immunogenic and nonabortogenic when ewes in the second third of pregnancy were inoculated with  $5 \times 10^5$  PFU of the viral strain. The ewes delivered live, healthy lambs that had no neutralizing antibody to RVFV until after they had ingested colostrum. To further assess the safety and protective capability of this candidate vaccine, 6 pregnant ewes were inoculated with  $5 \times 10^5$  PFU of MV P12 and challenged with  $5 \times 10^5$  PFU of virulent ZH-501 strain of RVFV 30 days later. No viremia was detected after vaccination or challenge and all 6 ewes delivered live, healthy lambs. Those lambs tested prior to their nursing colostrum did not have neutralizing antibody to RVFV but quickly acquired antibody titers of 1:320 to  $>1:10240$  after nursing colostrum. To test the safety of the MV P12 immunogen in neonates, lambs <7 days old, born to unvaccinated ewes, were inoculated with  $5 \times 10^5$  PFU of MV P12. With the exception of a brief pyrexia on the third day after inoculation and a transient low-titered viremia in 6 of 12 lambs, no untoward effects were observed. Serum-neutralizing antibody to RVFV was detected 5 to 7 days after inoculation. The results of these studies as well as those from tests in non-human primates support the potential of this viral strain as a live-attenuated vaccine for domestic animals and man.

TROPICAL MEDICINE COMMEMORATIVE FUND LECTURE:

359 PROGRESS IN THE TREATMENT AND PREVENTION OF ARGENTINE HEMORRHAGIC FEVERS. Julio I. Maiztegui. Director, Instituto Nacional de Estudio sobre Virosis Hemorragicas, Pergamino, ARGENTINA.

R: CLINICAL TROPICAL MEDICINE

- 360 CAMPYLOBACTER ENTERITIS ASSOCIATED WITH TAKING DOXYCYCLINE PROPHYLAXIS FOR MALARIA IN THAILAND  
D.N. Taylor,\* C. Pitarangsi, and P. Echeverria, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

Little is known about the untoward effects of doxycycline for malaria prophylaxis. During a 6 week long field training exercise in Thailand Campylobacter jejuni was isolated from 14 (50%) of 28 United States soldiers who developed a gastrointestinal illness while taking doxycycline (100 mg daily) for malaria prophylaxis. All C. jejuni isolates were resistant to tetracycline (MIC > 32 mcg), and susceptible to erythromycin and ciprofloxacin. Three soldiers were concomitantly infected with tetracycline-resistant Salmonella species and 1 soldier with enterotoxigenic Escherichia coli. The 14 C. jejuni isolates belonged to 6 different Lior serotypes, suggesting that they were from multiple sources. The illness associated with Campylobacter infections was characterized by abdominal pain (82%), diarrhea (73%), and fever (64%). Bloody diarrhea was observed in 2 (18%) of soldiers. Soldiers infected with C. jejuni more often had an oral temperature of > 38.3 C (101 F) and were more likely to have treated with antibiotics for their illness than soldiers who presented with diarrhea and had a negative culture for enteric pathogens. Campylobacter enteritis acquired in Thailand may be a severe illness in non-immune hosts and should be considered among persons with diarrhea who have been taking doxycycline for prophylaxis.

- 361 COST BENEFIT OF VACCINATION WITH Ty21a ORAL TYPHOID VACCINE IN PLAJU, INDONESIA. N.H. Punjabi<sup>1\*</sup>, P.P. Paleologo<sup>1</sup>, C.H. Simanjuntak<sup>2</sup>, Dicky A.S.<sup>3</sup>, and H.M.P. Choesni<sup>3</sup>. <sup>1</sup>U.S. NAMRU-2 Detachment, <sup>2</sup>NIHR&D Health Ministry, <sup>3</sup>Pertamina Oil Co., Indonesia.

From 25 Aug until 13 Sep 1986, employees of Pertamina Oil Co. (Plaju, Indonesia) and their families received Ty21a oral typhoid vaccine in a randomized, double blind, placebo controlled trial. Of the 22,001 participants in the trial, 20,543 received the recommended 3 doses of either enteric coated capsule vaccine (5,209), liquid/buffer (5,066) vaccine or placebo (10,268). During 18 months case detection surveillance, 2,439 blood cultures were obtained from trial participants; 1,193 were from vaccinees and 1,246 from the placebo group. Of 223 cultures positive for S. typhi, 81 were from vaccine recipients (liquid=38, capsule=43) and 142 from the placebo group. This represents an attack rate of 922/10<sup>5</sup>/yr in the placebo group, with a 40% protective efficacy for the capsule and 47% for the liquid. Thus, there were 61 cases of typhoid prevented overall in the vaccine recipient group. For cases, combined hospitalization and convalescence was 13 days. Mean hospital cost was US\$91+\$15; the hospital cost savings with vaccination were US\$5,551+\$915. At a purchase price over \$10,275 (\$1.00/person, liquid vaccine slightly more expensive x 10,275 vaccinees), vaccination was not monetarily cost effective in this population. This analysis does not take into consideration loss of productivity and complications due to infection. Since 186 (83.4%) cases were in children < 14 years of age, the impact on the work force was small. Only few complications were observed [melena (2), myocarditis (2), hepatitis (2) and bronchitis (1)]. There were 3 deaths, 1 placebo recipient and 2 non-participants. Final (2 year) data from this vaccine trial will be reported.

## R: CLINICAL TROPICAL MEDICINE

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J.P.DIGOUTTE, A.JOUAN, B.LE GUENNO, O.RIOU, B.PHILIPPE, F.ADAM, J.MEEGAN, T.G.KSIAZEK, and C.J.PETERS

Institut Pasteur DAKAR Senegal, Hôpital of Rosso Mauritania and USAMRIID FORT DETRICK

### RIFT VALLEY FEVER EPIDEMIC IN MAURITANIA

During the last two weeks of October 1987 a Rift Valley Fever outbreak occurred in the Trerza district, Mauritania Islamic Republic. More than three hundred cases were confirmed by virus isolation and/or by detection of specific IgM. The sensitivity of the various methods are discussed.

Among nearly three hundred confirmed clinical cases, jaundice and hemorrhagic manifestations were encountered in 16.7% with a mortality rate of 38.3%. The other form was fever with jaundice or with hemorrhage (38%) meningoencephalitis (5.7%) and the balance were uncomplicated forms.

Several epidemiological studies were carried out to estimate incidence rates in the different communities inside the epidemic area.

This epidemic occurred around the town of Rosso, along the Senegal River. We detected the virus : in nomad camps along the road between Rosso and Nouakchott, at 88 Km north of Rosso in Keur Massene, at 83 Km west of Rosso in Tessem and at 90 Km east of Rosso.

The epidemic wave seemed to be going from west to east during the 2 weeks of the acute phase.

The most common animals in this country are goats and sheep. About 80% of the animals samples contain specific IgM. Abortion rates of contaminated flocks were about 90%. Contamination of humans seems to result from close contact with animals.

Ecologic changes occurred for 2 years after the Makadiama dam was built.

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CLINICAL AND EPIDEMIOLOGIC CHARACTERISTICS OF AFRICAN TRYPANOSOMIASIS IN AMERICAN TRAVELERS. Ralph T. Bryan,\* H. Waskin, F. Richards, T. Bailey, D. Juranek, Parasitic Diseases Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.

In the past 2 decades (1967-1987), 14 American travelers were treated for African trypanosomiasis (*T. rhodesiense*) upon their return to the U.S. Eleven patients (79%) were short term travelers (2-28 days) participating in organized photographic or hunting safaris. Ages ranged from 19-74 (Mean=50). Of the 7 countries visited by patients, Tanzania (4 cases), Botswana (3 cases), and Rwanda (3 cases) were most frequently identified as countries of probable exposure. Presenting signs/symptoms included fever (100%), rash/skin lesions (76%), lethargy (64%), headache (43%), myalgia (36%), and confusion (36%). Incubation periods ranged from 6-28 days. Within 7 days of symptom onset, 92% of patients had seen a physician and a diagnosis was made within 4 days in 61% of cases. However, diagnostic delays in two cases (10 days and 15 days) after first physician visit were associated with CNS involvement. Overall, significant morbidity was common with thrombocytopenia, elevated hepatic transaminases, and anemia occurring in 69%, 69%, and 62% respectively. Renal insufficiency (31%), leukopenia (31%), hyponatremia (23%), cardiac arrhythmias (15%) and spurious hypoglycemia (7%) posed management problems. Two patients experienced DIC and 2 others developed severe hypoxia, but no deaths occurred. All patients were treated initially with suramin. Adverse reactions included transient rashes (5 patients), gastrointestinal complaints (1 patient), plantar paresthesias (1 patient), rigors (1 patient), and probable Jarisch-Herxheimer type reaction in 1 patient. One of the 2 patients receiving melarsoprol for CNS disease developed ataxia, weakness, and tremor suggestive of toxic encephalopathy.

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PLACEBO-CONTROLLED COMPARISON OF LOCAL HYPERTHERMIA AND GLUCANTIME (MEGLUMINE ANTIMONATE) IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS IN GUATEMALA.

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Sixty-six Guatemalans with parasitologically proven cutaneous leishmaniasis were randomly divided into 1 of 3 treatment groups: local hyperthermia from a radio-frequency generator that produced 50°C for 30 seconds to a depth of 4 mm; Glucantime, 850 mg per day IM for 15 days; and placebo treatment. Of 62 isolates speciated, 47 were L. braziliensis and 15 were L. mexicana. Thirteen weeks after treatment the number of patients in the 3 groups with completely healed and parasitologically negative lesions were: hyperthermia, 16 (73%) of 22; Glucantime, 17 (77%) of 22; and placebo treatment, 6 (27%) of 22. The response rates of both the hyperthermia and Glucantime groups are significantly greater ( $p < 0.01$ ) than that of the placebo group. The differences between treatment and placebo groups were even greater for infections due to L. braziliensis; the number of patients with healed and parasitologically negative lesions in this subgroup were: hyperthermia, 13 (72%) of 18; Glucantime, 14 (88%) of 16; and placebo treatment, 0 of 13. In summary, both hyperthermia and Glucantime are useful treatments of cutaneous leishmaniasis due to L. braziliensis in Guatemala.

365 CYSTIC HYDATID DISEASE: NEED FOR A REAPPRAISAL OF ASPIRATION CYTOLOGY FOR DIAGNOSIS ?

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A major drawback of serodiagnostic assays for cystic hydatid disease (CHD) is the significant number of false negatives. Direct demonstration of parasite elements is the ideal but this is contraindicated; a dangerous sequelae to the release in situ of the contents of a hydatid cyst being the reported initiation of an acute anaphylactic-like reaction. With the advent of ultrasound (US) and computerized tomography (CT), such imaging techniques are excellent at accurately localizing a mass. We report on US-guided aspiration of such lesions in serologically-negative patients or in whom CHD was not a differential diagnosis. No complications were observed that could be attributed to aspiration. The procedure may prove to be the primary method to diagnose serologically negative patients, especially in pulmonary CHD. Nuclepore filtration of the cyst fluid is quick and accurate, since the total volume may be examined. We highlight also the superiority of Trichrome compared to Hematoxylin and Eosin or Giemsa for staining protoscolices and hooklets. Thus, the historical aversion to aspiration of hydatid cysts may need a reappraisal. With the possibility of unequivocal diagnosis and an effective anthelmintic such as Albendazole, surgery may not be the only alternative or always necessary, pending the experience from other endemic areas.

R: CLINICAL TROPICAL MEDICINE

- 366 MALARIA PROPHYLAXIS WITH PROGUANIL/SULFONAMIDE IN THAILAND  
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Singharaj Armed Forces Research Institute of Medical  
Sciences APO S.F.96346

Adequate regimens for malaria prophylaxis have become more difficult as drug-resistant falciparum malaria has spread. The withdrawal of long-acting sulfa combinations as prophylactic agents due to parasite resistance and allergic reactions lead to the idea of trying proguanil in combination with short-acting sulfa drugs. Karen village children on the Thai-Burmese border were entered into a randomized unblinded placebo controlled clinical trial of daily malaria chemoprophylaxis after obtaining informed parental consent. Children were seen every day and all medication was given by the investigators.

Proguanil at an adjusted adult dose of 200 mg/day was given along with sulfisoxazole at 75 and 25 mg/kg and sulfamethoxazole at 25 and 10 mg/kg. Placebo was a multivitamin tablet. The series of three trials continued over 16 months with continuous medication being given for no longer than 4 months.

Combinations of proguanil/sulfisoxazole and proguanil/sulfamethoxazole were equally effective (>80%) chemoprophylaxis when the sulfa component was used at 25 mg/kg. Sulfamethoxazole at 10 mg/kg was ineffective. Approximately 1% of the children receiving sulfa drugs had related skin rashes which resolved on discontinuation of the medication.

This combination is an alternative malaria prophylactic regimen in multi-drug resistant areas of South East Asia.

- 367 CHLOROQUINE-INDUCED PRURITUS. A.U. Orjih\*, M.D. Ene, and D.J. Krogstad. University of Port Harcourt, NIGERIA and Washington University, St. Louis, MO.

The value of chloroquine as an antimalarial is compromised in most of Africa (and in Nigeria, in particular) by the syndrome of chloroquine-induced pruritus. This syndrome is often so distressing that persons with malaria refuse to take chloroquine even when they are severely ill. We performed these studies in southeastern Nigeria to estimate its prevalence, and to define its usual clinical features.

Questionnaires were administered to 113 Nigerian nursing and medical students because of their awareness of specific drugs. The results suggest that this syndrome may affect large numbers of adults (58% of those surveyed). Affected persons generally experienced pruritus within 24 hours of taking chloroquine, often within 6 hours. It typically began on the palms and soles, and progressed to involve the trunk, extremities, and perineal area. In most affected persons (89%), it then generalized and persisted for 2-3 days. All available brands of chloroquine were associated with the syndrome. Pruritus occurred if chloroquine was taken with or without symptoms of malaria or a positive smear. However, most persons surveyed did not take chloroquine unless they had symptoms of malaria. Antihistamines prevented the pruritus in 78% of affected persons, and relieved its symptoms in 47%. The syndrome was also found in 1 expatriate who had spent more than 10 years in Africa. These data suggest that chloroquine-induced pruritus is a widely prevalent syndrome, although its cause is unknown.

R: CLINICAL TROPICAL MEDICINE

- 368 EFFICACY AND SEVERE SIDE EFFECTS OF MEFLOROQUINE AGAINST FALCIPARUM MALARIA IN FRENCH TRAVELLERS. J.Le Bras, B.Rouveix, F.Simon, F.Bricaire, J.P.Coulaud. Nat. Reference Center for chemosensitivity of Malaria and INSERM U13; Hôp. Claude Bernard 75944 Paris France.

Mefloquine (Lariam®) is available in France since June 1986 for prophylaxis and treatment of chloroquine resistant *P. falciparum* (PF). Until April 1988, no failure was confirmed among approximately 300,000 therapeutic (25 mg/kg/1-2 days) or prophylactic (4mg/kg/week x6) mefloquine (MQ) regimens sold. Minor side effects (nausea, vomiting) were frequently noticed. Nevertheless, there is some evidence for 1) presence of MQ resistant PF in Africa and 2) association between occurrence of encephalitis and use of MQ.

1) Among 1273 PF cases imported from Africa into France whose strains were cultivated in National Reference Center in 1985-88, 387 were cured with mefloquine alone (n = 193, average dosage 23 mg/kg) or associated with quinine (n = 184) or other drugs (n = 10) and 436 were cured with chloroquine and/or quinine. For 96 patients treated with MQ, an in vitro isotopic semi-microtest was performed. The MQ IC<sub>50</sub> of the PF strains were between 1.7 and 28 nmol/l but one from Kenya (with MQ prophylactic failure of 2.7 mg/kg/7d followed by therapeutic success of a 25mg/kg MQ dose) was 40 nmol/l. The IC<sub>50</sub> predictive for in vitro resistance has been considered as 29 nmol/l (12ng/ml) or greater (1). For 263 patients cured with chloroquine and/or quinine and with assessment of the strain sensitivity to MQ, IC<sub>50</sub> were >28 nmol/l in 31. 21 strains of these 31 were originating from West Africa and were sensitive to chloroquine(2).

2) Four convulsions were observed in adults 2 to 19 days after MQ therapy. One other convulsion was observed in a 5 years child after prophylaxis. For the five patients, no parasitaemia and increase albuminorachia was found. MQ levels (HPLC) in serum and LCR were 3360, 1560, 6720 and <50, 415, 1160, µg/ml in subjects 1(d+2), 2(d+7), 5(d+15) respectively. One subject took antihypertensives and anticoagulant drugs, and one took quinine. Two cases reactivated troubles under quinine.

Due to these observations of in vitro decrease of sensitivity and of neurological toxicity, therapeutic and prophylactic usage of MQ must be done carefully.

1) Lancet, 1987, ii, 1304-05; 2) Lancet, 1988, i, 467-68

- 369 MALARIA PREVENTION IN TRAVELERS TO KENYA. Lobel H.O.,\* Brandling-Bennett A.D., Steffen R., Phillips-Howard P., and Were J.B.O. Malaria Branch, CDC, Atlanta, GA; Kenya Medical Research Institute, Nairobi; Institute of Preventive and Social Medicine, Zurich; London School of Hygiene and Tropical Medicine, London.

Malaria in travelers to Kenya has increased markedly during the past decade. To determine the use and safety of malaria prophylaxis a survey was conducted among 14,228 travelers from Nairobi to Europe. A total of 8543 (60%) travelers responded, including 2434 travelers from the U.S., 1940 from continental Europe, and 1803 from the U.K. A follow-up survey was conducted 10 weeks later.

The Kenya coast was visited by 55% of European but only 24% of U.S. travelers. Gameparks had been the sole destination for 22% of European and 60% of U.S. travelers. Over-all, 96% of travelers were aware of potential malaria risk and 91% had used chemoprophylaxis. More than 40 different drugs or drug combinations were used. Compliance with chemoprophylaxis ranged from 33% to 57% depending on nationality, age, purpose and length of travel, drugs used, and the occurrence of adverse drug reactions. Adverse reactions occurred in 10-18% of travelers. 70% of all travelers had used antimosquito measures. Only 32% of travelers carried drugs to treat a febrile illness. The difference between prophylaxis and presumptive therapy was unclear to many travelers. Only 1511 (22%) of travelers had been advised by travel agents to use chemoprophylaxis. The wide variety of chemoprophylactic regimens used and the poor compliance indicate that guidelines for malaria prevention need to be less complex and more uniform.

R: CLINICAL TROPICAL MEDICINE

- 370 MORTALITY DUE TO IMPORTED PLASMODIUM FALCIPARUM MALARIA IN UNITED STATES TRAVELERS: 1959-1987. A.E. Greenberg,\* H.O. Lobel. Malaria Branch, CDC, Atlanta, GA.

To characterize mortality due to imported *P. falciparum* malaria in the United States, we reviewed the 67 deaths in U.S. travelers that were reported to the CDC between 1959 and 1987. Overall, 72% of the patients with fatal *P. falciparum* infections were >40 years old (mean age 49 years), 64% were males, and 78% acquired their infections in Africa. The leading reasons for travel were tourism (50%), missionary work (21%), and business (17%). Only 24% of patients with fatal infections had used chemoprophylaxis, compared to 45% of travelers with non-fatal *P. falciparum* infections in 1981-1984 ( $p=.01$ ). All but 2 patients developed malaria-related symptoms within 2 weeks after returning to the United States. The mean duration between onset of symptoms and initial visit to a physician was 5.7 days. The most common presenting symptoms were fever/chills (89%), malaise/weakness (55%), nausea/vomiting/diarrhea (47%), altered mental status (36%), myalgias (22%), and headache (19%). Nine patients (13%) were classified as dead on arrival. In 34% of the fatal cases, the diagnosis of malaria was not made by the physician during the initial visit, resulting in a delay in therapy. The overall case fatality rate of *P. falciparum* malaria in U.S. travelers between 1966 and 1985 was 4.4%; the age-specific rate increased progressively with age, from <1% in patients <20 years old to 8.9% in patients >50 years old ( $p<.001$ , Chi-square test for trend). These data indicate that failure to take chemoprophylaxis, delay in seeking medical attention, misdiagnosis by physicians, and older age contribute to a fatal outcome in *P. falciparum* malaria in U.S. travelers.

- 371 REASSESSMENT OF BLOOD DONOR SELECTION CRITERIA FOR U.S. TRAVELERS TO MALARIOUS AREAS. B.L. Nahlen,\* S.E. Cannon, and C.C. Campbell. Malaria Branch, CDC, Atlanta, GA.

To prevent transfusion-induced malaria (TM) due to two relapsing forms of malaria, *Plasmodium vivax* and *P. ovale*, U.S. travelers who have had malaria or who have taken antimalarial chemoprophylaxis are deferred from donating blood for 3 years. To assess the impact of shortening this 3-year exclusion period, national malaria surveillance data from 1972 to 1987 were reviewed. The average annual rate of TM is 0.25 cases per million units collected. Of 44 reported TM cases, 36% were caused by *P. malariae*, 32% by *P. falciparum*, 25% by *P. vivax* and 7% by *P. ovale*. Thirty donors were implicated in 32 cases of TM. Of implicated donors, 23 (77%) were foreign nationals and 7 (23%) were from the U.S.A. In a review of all imported malaria cases by species and by interval between date of entry and onset of illness, 96% of *P. falciparum*, 84% of *P. malariae*, 79% of *P. ovale* and 76% of *P. vivax* infections became symptomatic within 6 months of arrival in the U.S. *P. malariae* and *P. falciparum* are responsible for two-thirds of TM cases. U.S. travelers, who account for only one-quarter of TM cases, will become symptomatic within 6 months of arrival in the U.S., regardless of prophylaxis use. Shortening the donor-exclusion period for U.S. travelers to malarious areas to 6 months would result in a minimum 56,250 additional blood donors made available, with a maximum increase of 0.24 additional cases of TM annually. CDC is cooperating with the blood banking industry to systematically evaluate whether or not the benefit of bringing healthy travelers back into the donor pool is worth the increased risk of TM.

R: CLINICAL TROPICAL MEDICINE

- 372 THE SITES, SOUNDS AND SYMPTOMS OF DERMATOBIA HOMINIS:  
A STEREO SCAN, MOTION AND ELICITED HOST RESPONSE STUDY  
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The heiroglyphic sign of a fly stands for "imputence" and "courage", best illustrated by the unique ability of the gravid human botflies, largely oestridae family, to glue their eggs to other vectors while in fugative hovering flight and thus create a subsequent larval residence in the human host. This facultative myiasis life cycle is exemplified by a Graduate student's infection acquired during a brief stay in Guatemala. The clinical presentation and the excruciating host pain elicited during larval movements are described along with motion films of the larval respiratory action within the host's lesion.

The cutaneous furuncular ulceration, the excised larval gross appearance, its topographic characteristics as seen by stereoscan electron micrography and the associated clinical correlations of the travel history, diagnostic signs and unusual treatments will be presented.

S: MALARIA - SPOROZOITES

373 IMMUNIZATION OF HUMAN VOLUNTEERS WITH A RECOMBINANT *PLASMODIUM FALCIPARUM* SPOROZOITE VACCINE, R32NS1<sub>81</sub>

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Protection from experimental *Plasmodium falciparum* (Pf) sporozoite challenge has previously been achieved by immunization with the recombinant vaccine, R32tet<sub>32</sub>, but this vaccine was not sufficiently immunogenic for general use. In order to enhance immunogenicity, a second recombinant fusion protein was produced in which the 32 amino acid tet<sub>32</sub> portion of the molecule was replaced with an 81 amino acid fragment of the nonstructural protein 1 of Influenza A (R32NS1<sub>81</sub>). Alum adsorbed R32NS1<sub>81</sub> was administered to 15 human volunteers at 11.5, 115, or 1150 µg per dose at weeks 0, 8, 16 and 23. The vaccine was well tolerated and induced IgG antibodies against Pf antigen by ELISA in all 15 volunteers. Response was not dose-related. Antigen-specific IgG levels ranged from 1.0 µg/ml to more than 60 µg/ml after three doses were administered. One volunteer (115 µg group) had a marked booster response to the third dose (>100-fold increase in ELISA titer). At week 25, 10 immunized subjects and 2 unimmunized controls were challenged by the bite of Pf-infected mosquitoes. Delayed patency (1-2 days) was observed in 8 immunized subjects, but all volunteers developed patent parasitemia. As measured by ELISA, immunofluorescence, and inhibition of sporozoite invasion *in vitro*, several volunteers had antibody responses similar to that of the volunteer protected after immunization with R32tet<sub>32</sub>. These results emphasize that the currently available serologic tests fail to predict protective immunity.

374 ESTIMATE OF *PLASMODIUM FALCIPARUM* SPOROZOITE CONTENT OF *ANOPHELES STEPHENSI* USED TO CHALLENGE HUMAN VOLUNTEERS.

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Dissection and microscopic examination and a radioimmuno-metric procedure were used to estimate sporozoite content of mosquitoes which were part of a group used to challenge recipients of (NANP)<sub>3</sub>-TT vaccine and nonvaccinated controls. The microscopic procedures showed 10/10 mosquitoes infected with a mean gland index of 3.25. Radioimmuno-metric assay showed 13 sporozoite positive mosquitoes (e.g. greater than 48 sporozoite equivalents/mosquito) in a sample of 17 (76%); the mean sporozoite content of infected mosquitoes was 220,994 (range 141,247 to 300,000). Nonimmune individuals challenged with mosquitoes from this group developed patent infections at an average of 7.4 days after mosquito bites, near the minimum interval reported for patency for *P. falciparum* in humans. The pattern of infection in volunteers and the magnitude of mosquito infections suggest that challenges prepared under laboratory conditions may be more formidable than those which occur in nature.

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375 SAFETY AND IMMUNOGENICITY OF CONJUGATE PLASMODIUM FALCIPARUM SPOROZOITE VACCINES USING SYNTHETIC OR RECOMBINANT ANTIGENS COUPLED TO CHOLERA TOXIN AND PSEUDOMONAS TOXIN A.

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Highly immunogenic Plasmodium falciparum sporozoite vaccines will be required to protect humans against malaria. We synthesized a series of experimental vaccines by covalently coupling a recombinant circumsporozoite (CS) repeat protein (R32), or synthetic CS peptides (NANP)3, (R3) or (NANP)6, (R6) to the carrier proteins cholera toxin (CT) or Pseudomonas toxin A (TA). CT and TA were derivatized by coupling adipic dihydrazide (ADH) to them using water soluble carbodiimide (EDC). Next, R3, R6, or R32 were conjugated to the carriers via the ADH spacer molecules using EDC. For two preparations, R32 was derivatized with succinic anhydride (R32S) to convert free amino groups to carboxyl moieties prior to conjugation to CT or TA. Molar ratios of peptide:carrier varied from 1:1 to 7:1 for conjugates containing the recombinant protein (R32SCT < R32STA < R32CT < R32TA), and were 25:1 and 33:1 for those containing synthetic peptides, R3CT and R6CT respectively. All six vaccines were nonpyrogenic and nontoxic, and when adsorbed to alum were highly immunogenic for rabbits. Human volunteers (4-5/group) received two 100 ug (carrier protein) doses at weeks 0 and 8. The vaccines were well tolerated, but marked differences in immunogenicity were observed. Seroconversion (four-fold antibody rise by ELISA) was observed in 4/5 volunteers receiving R32TA, and in 3/5 receiving R3CT or R32STA. In contrast, no responses were seen in any volunteer receiving R32CT, R6CT and 3/4 receiving R32SCT. In general, ELISA titers correlated with both immunofluorescent assay and in vitro inhibition of sporozoite invasion. Seroconversion to CS epitopes was independent of antibody responses to carrier proteins. TA appears to be a superior carrier protein for conjugate P. falciparum sporozoite vaccines.

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HLA-DR AND LYMPHOCYTE RESPONSIVENESS TO THE CIRCUMSPOROZOITE PROTEIN OF PLASMODIUM FALCIPARUM.

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Extensive efforts are underway to develop a human vaccine directed against malaria sporozoites, and particularly the circumsporozoite (CS) protein, of the parasite surface. In mice, both humoral and cellular responses to epitopes in the central repeat region of the CS protein from Plasmodium falciparum have been found to be restricted by the major histocompatibility complex. In order to determine if similar T cell restriction may also be present in humans, Thai Rangers who had experienced falciparum malaria while deployed in malaria endemic areas along the Thai-Kampuchean border were studied. Both humoral and cellular responses to R32tet32 were determined, and B lymphocytes were serologically typed for Class II HLA phenotypes. Significant associations were found between both DR5 and DRw52 and lymphocyte non-responsiveness. These results suggest that genetic restriction may render some humans unable to mount a T cell response to P. falciparum sporozoites and, presumably, to sporozoite-derived vaccines.

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- 377 PLASMODIUM FALCIPARUM CS VACCINES ELICIT ANTIBODIES IN HUMAN VOLUNTEERS THAT INHIBIT SPOROZOITE INVASION OF HEPATOMA CELLS BUT ENHANCE INVASION OF HUMAN HEPATOCYTES. M. R. Hollingdale,\* D. Mazier, A. Appiah, P. Leland, T. Derks, W. R. Ballou, D. A. Herrington, S. H. Yap, J. P. Verhave. Biomedical Research Institute, Rockville, MD., CHU Pitie-Saltpetriere, Paris, France; U. Nijmegen Medical School, The Netherlands; WRAIR, Washington, D.C., University of Maryland School of Medicine, Baltimore, Md.

Immunization of mice and rabbits with recombinant protein (R32tet<sub>32</sub>) and synthetic peptide (NANP)<sub>5</sub> vaccines elicited antibodies that inhibited P. falciparum sporozoite invasion (ISI) of human hepatoma (HepG2-A16) cells and human hepatocytes (HH). Sera from the first human volunteer trials with R32tet<sub>32</sub> and (NANP)<sub>3</sub>-TT were compared for ISI using HepG2-A16 and HH cells. Immunoglobulins Ig of pre-immunization and pre-challenge sera were partially purified and dilutions added to cell cultures before the addition of standard numbers of P. falciparum sporozoites. Invaded sporozoites were counted microscopically. ISI<sub>50</sub> was that concentration of immune Ig that reduced invasion by 50% compared to pre-immunization Ig. Using HepG2-A16 cells, Ig from either protected volunteers, or who had extended prepatent periods, blocked invasion (ISI<sub>50</sub> = 2-10µg/ml), whereas Ig from non-protected volunteers were much less active (ISI<sub>50</sub> = 42->100µg/ml). In contrast, sera from immunized volunteers enhanced (1.5-4.0 times) P. falciparum invasion of HH cells. Implications for the use of functional assays in sporozoite vaccine trials will be discussed. This study was supported by the availability of sera from WRAIR and U. Maryland, and partially by AID contract DPE-0453-C-00-3051-00.

- 378 PLASMODIUM FALCIPARUM SPOROZOITES FROM MOSQUITOES PREVIOUSLY FED ANTI-NANP ANTIBODIES ARE NO LONGER NEUTRALIZED BY SERA FROM A HUMAN VOLUNTEER IMMUNIZED WITH R32TET<sub>32</sub> VACCINE AND PROTECTED TO CHALLENGE. M.R. Hollingdale,\* A. Appiah, J. Vaughan and V.E.do Rosario. Biomedical Research Institute, Rockville, MD. and School of Medicine, University of Maryland, Baltimore MD.

We have previously shown (Vaughan et al Exp. Parasitol. 1988) that P. falciparum-infected Anopheles stephensi mosquitoes fed rat anti-P. falciparum sporozoite (RSPZ) or rabbit anti-R32tet<sub>32</sub> (RR32) antibodies show enhancement of the numbers of sporozoites recovered from salivary glands. Further experiments were performed to test whether sporozoites from immune fed mosquitoes were altered in their susceptibility to neutralization with anti-circumsporozoite (CS) protein antibodies. A. stephensi mosquitoes were infected with P. falciparum gamatocytes, and 5 days later fed RSPZ or RR32 antibodies. Sporozoites were recovered from immune fed and control mosquitoes and tested in the inhibition of sporozoite invasion (ISI) assay using serum from a human volunteer (14) immunized with R32tet<sub>32</sub> vaccine and immune to P. falciparum challenge (Ballou et al Lancet 1987) or Mab 2A10 directed to CS protein. Serum 14 readily neutralized control sporozoites with an ISI<sub>50</sub> of about 2 µg/ml, but had little effect on sporozoites from immune-antibody fed mosquitoes even at 100 µg/ml. In contrast, the ISI<sub>50</sub> of Mab 2A10 against control sporozoites was 1 µg/ml and against immune-fed sporozoites was 4 µg/ml. The reasons for these differences may be explained by either a) selection of a parasite line or lines that are less susceptible to neutralization, or b) changes in conformation or expression of immunodominant epitopes induced by feeding anti-CS antibodies during sporogony. This work was supported by AID contract DPE-0453-C-00-3051-00 (MH) and WHO # 870105 (VR).

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379 HUMORAL AND CELLULAR IMMUNE RESPONSES IN VOLUNTEERS IMMUNIZED WITH IRRADIATED *P. FALCIPARUM* SPOROZOITES.

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Early studies carried out in the 70's clearly demonstrated that man could be protected against malaria after immunization by multiple exposures to the bites of irradiated Plasmodium infected mosquitoes. The cloning of the CS gene in 1984 has resulted in the production of recombinant proteins and synthetic peptides which can now be utilized to study cell mediated immunity in sporozoite immunized individuals.

Four volunteers were exposed to the bites of irradiated *P. falciparum* (NF54) infected Anopholes mosquitoes. Three of the four volunteers had positive ant sporozoite antibody IFA titers following two exposures to a total of 33 - 88 irradiated infected mosquitoes. PBL obtained from the three seropositive individuals proliferated when challenged *in vitro* with a recombinant *P. falciparum* CS protein which contains approximately 70% of the total CS protein. PBL of the individuals exposed three times to the bites of irradiated *P. falciparum* infected mosquitoes did not proliferate when challenged with synthetic peptides representing the repeat region of *P. falciparum* or with peptides representing regions of the CS protein with predicted amphipathic alpha helix secondary structures. Clonal analysis of the T cell response is being carried out using a rPfCS-1 specific T cell line obtained from one of the sporozoite immunized volunteers.

Work supported by A.I.D. Grant No. DPE-0453-A-00-5012-00.

380 PRESENTATION OF PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN EPITOPES ON THE SURFACE OF RECOMBINANT HEPATITIS B SURFACE ANTIGEN TO ENHANCE IMMUNOGENICITY OF A SPOROZOITE VACCINE.

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Malaria sporozoite vaccines must induce high levels of antibody to confer protective immunity. Although the repeat portion of the *Plasmodium falciparum* (Pf) circumsporozoite (CS) protein contains B cell epitopes critical to protection, this region has been shown to be lacking in human helper T cell epitopes. To exploit the numerous T cell epitopes present on the highly immunogenic HBsAg protein, we expressed, in *Saccharomyces cerevisiae*, a hybrid protein containing [(NANP)<sub>15</sub>(NVDP)<sub>1</sub>] from the Pf CS protein fused to a 42 amino acid sequence from the pre-S2 region and 226 amino acids from the HBsAg. Cesium chloride equilibrium centrifugation and electronmicrographs demonstrate that this hybrid protein (R16HBsAg) is assembled into a particle similar to native HBsAg. These hybrid particles expose the CS epitope on their exterior based on their ability to react with anti-CS antibodies when bound to solid phase anti-HBsAg. Alum-adsorbed R16HBsAg was extremely immunogenic for mice, rabbits, and monkeys. C57Bl/6 mice primed with HBsAg developed anti-CS antibody titers 5x higher (1:60,000) than unprimed controls following immunization with 1 ug of R16HBsAg. Conversely, mice primed with R16HBsAg had marked booster responses to CS epitopes following immunization with HBsAg alone. We speculate that the enhanced immunogenicity of this vaccine is due to the particulate nature of the hybrid protein and its excellent repertoire of helper T cell epitopes.

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381 PLASMODIUM FALCIPARUM SPOROZOITES PROTECT MICE TO CHALLENGE WITH P. BERGHEI SPOROZOITES. V. E. Rosario,\* J. Vaughan, S. Aley, G. Woollett, and M. R. Hollingdale. Biomedical Research Institute, Rockville, MD., and School of Medicine, University Maryland, Baltimore MD.

Irradiated Plasmodium berghei sporozoites have been previously shown to protect mice against challenge with live P. berghei sporozoite challenge. As part of a study to define other sporozoite antigens as candidate vaccines, the immunological response of mice immunized with P. falciparum sporozoites was analyzed by challenge with live rodent sporozoites. Groups of 5-10 outbred mice were immunized biweekly 3-4 times by bite of P. falciparum-infected Anopheles stephensi mosquitoes when their IFA titer to P. falciparum sporozoites was >1:2,000. Mice were then challenged by bite of P. berghei-infected A. stephensi mosquitoes. In five experiments, 22/37 (60%) mice were completely protected (range 40-88%), compared to 100% of mice immunized with irradiated and challenged with live P. berghei sporozoites. Balb/c mice immunized intravenously with P. falciparum sporozoites were also protected to P. berghei challenge. Protection was specific to P. berghei as mice similarly immunized with P. falciparum were not protected to challenge with P. yoelii sporozoites. P. falciparum CS protein was unlikely to mediate this protection, as mice immunized with R32tet<sub>32</sub> were not protected to P. berghei sporozoites. However, sera from P. falciparum-immunized mice weakly fluoresced with P. berghei sporozoites. Such sera are being used to further characterize the antigen common to P. falciparum and P. berghei sporozoites that mediates protection. Thus this system provides a model in mice where protection to challenge by immunization with P. falciparum antigens may be studied. Supported by AID contract DPE-0453-C-00-3051-00.

382 MAPPING OF T CELL EPITOPES WITHIN THE P. BERGHEI CS PROTEIN  
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Using a yeast-derived CS protein of P. berghei, we identified and characterized a helper T cell epitope located in Region I, adjacent to the amino-terminal flank of the repeat domain of the CS protein. We also found that the main repetitive B cell epitope fails to have T cell stimulating activity.

We now report the identification of additional T cell epitopes located in the non-repeat containing amino- and carboxy-terminal regions of the CS protein. They are not present in the recombinant CS protein currently available. These other T cell epitopes were identified by immunizing mice with a series of synthetic CS peptides, followed by in vitro stimulation of their T cells with the corresponding synthetic peptides. The T cell response to most of these synthetic peptides is genetically restricted. However, one T epitope in the carboxy-terminal region of the CS protein appears to be recognized by T lymphocytes of all the different strains of mice we have tested. The functional activity of some of these T cell epitopes in the context of ant sporozoite immunity, will be the subject of discussion.

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383 PROTECTION OF MICE AGAINST PLASMODIUM YOELII SPOROZOITE-INDUCED MALARIA BY PASSIVE TRANSFER OF A MONOCLONAL ANTIBODY TO A DEFINED EPITOPE.

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Mice immunized with the Plasmodium yoelii irradiated sporozoite vaccine are solidly protected against a challenge with  $10^4$  sporozoites. *In vivo* depletion of CD8+ lymphocytes from immune mice eliminates resistance to challenge with 200 sporozoites, indicating that antibodies elicited by the irradiated sporozoite vaccine are inadequate to protect these mice against even a minimal sporozoite challenge. Nonetheless, monoclonal antibodies (Mabs) against P. yoelii sporozoites neutralize sporozoite infectivity. We therefore undertook studies designed to further define this observation using a standardized passive transfer test system. NYSI (Navy Yoelii Sporozoite I), an IgG<sub>3</sub> Mab was passively transferred into Balb/c mice and 30 min later the mice were bled and challenged with 200 sporozoites. Passive transfer of 500 µg of Mab NYSI was required to achieve 100% protection (6/6) against the 200 sporozoite challenge. This dose of antibody also protected 100% of mice (6/6) against challenge with  $5 \times 10^3$  sporozoites, and protected 67% of mice (4/6) against a challenge of  $2.5 \times 10^4$  sporozoites. NYSI recognizes an epitope in the major repeat domain of the P. yoelii CS protein (Gln-Gly-Pro-Gly-Ala-Pro). At the time of sporozoite challenge levels of antibodies to this epitope in mice receiving 500 µg of NYSI were between 2-4 times higher than levels found in vaccinated mice which failed to resist challenge with 200 sporozoites in the CD8+ depletion experiment, and in unprotected mice in subunit vaccine studies. There may be important differences between antibodies from these groups of mice regarding affinity and subclass. However, if the absolute level of antibody proves to be a crucial factor in antibody mediated resistance, levels of antibody 2-4 times higher than obtained with current vaccines will be required to achieve protective immunity.

384 COMPLETE PROTECTION AGAINST PLASMODIUM YOELII MALARIA BY IMMUNIZATION WITH A RECOMBINANT CS PROTEIN VACCINE.

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Initial attempts to immunize mice against Plasmodium yoelii with subunit vaccines based on the CS antigen failed to induce a solid protective response. These results followed a similar experience encountered in Phase II trials in humans vaccinated against P. falciparum. In the present study, a recombinant fusion protein containing about 60% of the P. yoelii CS protein was used to vaccinate mice. Briefly, groups of 6 Balb/c mice were immunized intramuscularly with 50, 100, 250, or 500 µg of recombinant protein. The first immunizing dose of vaccine was emulsified in complete Freund's adjuvant, followed at 2 week intervals by vaccine in incomplete Freund's adjuvant. Antibody levels as measured by IFAT against whole sporozoites and solid phase ELISA against various synthetic and recombinant peptides reached a plateau after 4 doses of vaccine, and levels correlated with antigen dose. Two weeks after administration of a fifth dose, all mice were challenged by iv administration of 200 P. yoelii sporozoites. All 10 non-immunized control mice, 33% of mice receiving 50-250 µg doses of vaccine, and none of the 6 mice that received the 500 µg dose developed parasitemia. This is the first report of successful induction of protection against P. yoelii malaria with a subunit vaccine. Studies are underway to determine the relative contributions of humoral and cellular mechanisms to this protective immunity.

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HELPER T CELLS ARE REQUIRED DURING THE INDUCTION OF CELL-MEDIATED  
385 IMMUNITY TO MALARIA SPOROZOITES.

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In mice fully immunized with *Plasmodium yoelii* sporozoites, depletion of CD4+ (helper) T cells does not alter immunity to sporozoites, but depletion of CD8+ (cytotoxic/suppressor) T cells leaves the animals unprotected. We wished to understand the development of these CD8+ effector cells, particularly the need for CD4+ helper cells during their induction by sporozoites.

BALB/C mice were depleted of CD4+ cells by i.p. injection of an anti-CD4 Mab. These mice were then immunized with two doses of irradiated *P. yoelii* sporozoites, and challenged with 5000 viable sporozoites. Control mice received normal immunoglobulin injections instead of Mab. Control mice were solidly protected against sporozoite infection, but mice treated with the anti-CD4 Mab were not immune.

T helper cells are thus required for the induction of effector T cells by irradiated sporozoites. Any vaccine which hopes to mimic this cell-mediated immunity must contain T epitopes for both CD4+ helper and CD8+ effector T cells.

## T: AMEBIASIS AND GIARDIASIS

- 386 CYSTEINE PROTEINASE EXPRESSION AND PATHOGENICITY OF ENTAMOEBA HISTOLYTICA. S.L. Reed\*, W.E. Keene, and J.H. McKerrrow, UCSD Medical Center, San Diego, CA and UCSD Medical Center, San Francisco, CA.

10% of the world's population is infected by Entamoeba histolytica (Eh), yet the factors that contribute to the resulting severity of infection are poorly understood. The major extracellular proteinase of axenic strains of Eh is a 56 kD neutral cysteine proteinase (CP-56) which can degrade extracellular matrices, collagen, plasminogen, and activate complement by specific cleavage of C3. In addition, production of the proteinase appears to correlate with the virulence of axenic strains. Because axenic strains have lost their virulence to a variable extent, we investigated the release of CP-56 by clinical isolates which had been characterized as pathogenic (P) or nonpathogenic (NP) based on zymodemes and clinical symptoms of the patients. All assays were performed on the supernatants of PBS containing  $10^7$  trophozoites/ml. Production of the proteinase was evaluated on 10% polyacrylamide gels copolymerized with 0.1% gelatin under nondenaturing conditions. Proteolytic degradation at a MW of 56 kD was found in 9 of the 9 pathogenic strains tested, but in only 1 of 10 NP strains. Total cysteine proteinase activity was quantified spectrofluorometrically by the cleavage of a synthetic peptide substrate, Boc-arginine-arginine-4-amino-7-methylcoumarin. Comparing 6 P and 10 NP strains, a clear trend towards greater proteinase production by pathogenic strains was seen. This proteinase is also released during the course of clinical invasive amebic disease as demonstrated by the presence of circulating antibodies to the 56 kD proteinase in patients with amebic colitis (N=9) or liver abscesses (N=15), but not in asymptomatic cyst carriers (N=8) or controls (N=20). These observations suggest that release of CP-56 correlates with the potential to cause invasive amebic disease.

- 387 MUCUS SECRETAGOGUE ACTIVITY OF ENTAMOEBA HISTOLYTICA IN RAT COLONIC LOOPS. K. Chadee, D.J. Innes, and J.I. Ravdin. Institute of Parasitology, McGill Univ., Canada, and Univ. of Virginia School of Medicine, Charlottesville, VA

Depletion and dissolution of the protective colonic mucus blanket occurs prior to in vivo invasion by Entamoeba histolytica (Eh). We studied the ability of Eh to stimulate mucus secretion in a rat colonic loop model. Following intraperitoneal injection of  $^3\text{H}$  or  $^{14}\text{C}$ -glucosamine, mucus secretion as quantitated by acid precipitation of  $^3\text{H}$ -labeled luminal proteins was increased in dose dependent fashion in response to  $\geq 10^5$  amebae. Cholera toxin (CT) (20 ug per loop) as a positive control elicited a similar secretory response. Thin section histology demonstrated depletion of apical mucosal goblet cells with mucus release, streaming, and cellular cavitation. Cell sloughing in response to Eh or CT did not occur as determined by histology or release of cellular DNA labeled with  $^3\text{H}$ -thymidine. Sepharose 4B column chromatography of luminal  $^3\text{H}$ -labeled glycoproteins revealed that Eh stimulated secretion of both high molecular weight ( $>9 \times 10^2$  Kd) void volume mucins and lower molecular weight glycoproteins found in included fractions. Eh and CT enhanced secretion of both pre-formed and newly synthesized mucin glycoproteins and stimulated glycoprotein synthesis as determined by incorporation of  $^{14}\text{C}$ -glucosamine into mucosal tissues. The level of mucus secretion elicited by different axenic Eh strains correlated with their in vivo and in vitro virulence. The secretagogue was released by Eh into the culture medium, was heat stable, and not inhibited by control or immune human serum. Eh mucus secretagogue activity may contribute to depletion of the protective colonic mucus blanket and thus pathogenesis of invasive amebiasis.

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- 388 BINDING OF THE ENTAMOEBA HISTOLYTICA GAL/GALNAC INHIBITABLE ADHERENCE LECTIN TO GLYCOSYLATION DEFICIENT CHINESE HAMSTER OVARY CELL MUTANTS. J.I. Ravdin, P. Stanley, C.F. Murphy, and W.A. Petri, Jr., Univ of Virginia School of Medicine, Charlottesville, VA and Albert Einstein College of Medicine, Bronx, NY.

E. histolytica (Eh) adherence (adh) to Chinese hamster ovary (CHO) cells is mediated by an amebic galactose (Gal) and N-acetyl-D-galactosamine (GalNAc)-binding lectin. CHO cell glycosylation mutants provide a means to further understand Eh adh. Adh of amebae to wild type (Gat<sup>-2</sup>) CHO cells was 65% as measured by a rosette assay. Eh adh to Lec 1 CHO cells (which express Man<sub>5</sub> oligomannosyl N-linked carbohydrates) was only 12%. Eh adherence to Lec2 and Lec3 CHO cells, whose glycoproteins and glycolipids have decreased terminal sialic acid exposing subterminal Gal, was increased to 92% and 87%. Neuraminidase treatment of Gat<sup>-2</sup> CHO cells also increased Eh adh to 89%. Amebic adh was decreased to 43% in Lec 8 CHO cells which are reduced in both terminal Gal and sialic acid, to 50% in Lec 4 CHO cells lacking  $\beta$  1-6 branching on core mannose residues, and to 36% in Lec 12 CHO cells that have  $\alpha$  1-3 linked fucoses added to non-sialylated lactosamines. However, addition of ( $\alpha$ 1-3) fucose to sialylated as well as non-sialylated lactosamines (Lec 11) increased adh to 75%. Using a membrane fraction of Eh, direct binding of the Gal/GalNAc lectin to mutant CHO cells was determined by indirect immunofluorescence and reflected the results of adh with intact trophozoites. Extracellular cytolysis by Eh was reduced for Lec 1 and increased for Lec 2 CHO cells compared to the Gat<sup>-2</sup> wild type. The Eh Gal/GalNAc adh lectin binds to Gal or GalNAc terminal CHO cell glycoprotein receptors; removal of terminal sialic acid residues from CHO cell receptors promotes lectin binding and thus parasite adh and cytolytic activities.

- 389 CHINESE HAMSTER OVARY CELLS DEFICIENT IN N-ACETYLGLUCOSAMINYL-TRANSFERASE I ARE RESISTANT TO E. HISTOLYTICA MEDIATED CYTOTOXICITY. E. Li\*, A. Becker, and S.L. Stanley, Jr. Washington University School of Medicine, St. Louis, MO 63110.

In order to study the relationship between carbohydrate specific amebic cytheadherence and amebic mediated cytotoxicity, we have measured E. histolytica trophozoite mediated cytolysis directed against a panel of lectin-resistant Chinese hamster ovary cell lines, which have defined alterations in their glycosylation patterns. We have previously shown with this panel of cells that amebic adherence corresponds with the presence of terminal N-acetyllactosamine units on the cells. Under certain conditions of a <sup>51</sup>Cr release assay, RIC<sup>R</sup> 15B cells, which have a selective deficiency in Asn-linked complex (N-acetyllactosamine) units and a corresponding increase in Asn-linked oligomannosyl units in its glycoproteins due to a deficiency in N-acetylglucosaminyltransferase I activity, were resistant to trophozoite mediated cytolysis compared with wild type cells. However susceptibility of two other lectin-resistant cell lines to amebic mediated cytotoxicity did not correspond to the results of adherence assays. We also noted that trophozoite motility was reduced in trophozoites incubated with RIC<sup>R</sup> 15B cells compared with wild type cells. These studies indicate that although surface carbohydrates on target cells influence susceptibility to amebic mediated cytotoxicity, the role played by surface glycoconjugates is complex and may not be simply related to the phenomenon of carbohydrate specific adherence. This panel of lectin-resistant CHO cells provides a useful model system for investigating the role of glycoconjugates in mediating amebic interactions with mammalian cells.

- 390 OLIGONUCLEOTIDE PROBES TO THE CELL SURFACE ADHERENCE LECTIN OF ENTAMOEBIA HISTOLYTICA. B.J. Mann, T. Snodgrass, E.L.W. Kittler, J.I. Ravdin, and W.A. Petri, Jr. Departments of Medicine and Microbiology, University of Virginia, Charlottesville, VA 22908.

The adherence of E. histolytica trophozoites to human colonic mucus, colonic epithelial cells and other target cells is mediated by a galactose (Gal) and N-acetyl-D-Galactosamine (GalNAc)-inhibitable surface lectin. The purified Gal/GalNAc adherence lectin competitively inhibits adherence to target cells in vitro and is the major amebic antigen recognized by human immune sera. The purified Gal/GalNAc lectin consists of a 170 kDa heavy chain and three 35 kDa light chains linked together by disulfide bonds. The first 15 amino terminal amino acids of the heavy subunit of the Gal/GalNAc lectin have been sequenced and three partially overlapping negative strand oligonucleotides synthesized based on this protein sequence. The oligonucleotide probes hybridized on Northern blots to a 4.0 kb RNA that was distinct from ribosomal RNA. The 4kb size of the mRNA identified by the oligonucleotides contains coding capacity sufficient for the heavy subunit of the Gal/GalNAc lectin. Oligonucleotide probes to aminoterminal or internal protein sequences will be used to isolate cDNA and/or genomic clones of the heavy subunit of the Gal/GalNAc lectin from libraries we have constructed. The sequence of the heavy subunit clone will provide the first step toward identifying functional and antigenic domains of the adherence protein.

- 391 HYBRID FORMATION IN ENTAMOEBIA HISTOLYTICA. E. Orozco\*, F. Solis, M.A. Vargas. Department of Genetics. CINVESTAV I.P.N. Apdo Postal 14-740, Mexico 07000, D.F.

Transference of DNA to Entamoeba histolytica was carried out by polyethyleneglycol fusion of two clones with different phenotypes. Clone C9, strain HMI:IMSS was the donor. This clone is emetine-resistant, highly phagocytic and virulent, and it is able to grow in semisolid agar. Clone L-6, strain HMI:IMSS, was the recipient. This clone is emetine-sensitive, phagocytosis- and virulence-deficient, and it does not grow in semisolid agar. Clone C9 was grown in 20 ug/ml bromodeoxyuridine for 24h. Cells then were irradiated with 310 nm light to complete inactivation. Controls were carried out to ensure that all the irradiated trophozoites died after 24 to 48 h incubation at 37°C. Irradiated trophozoites were fused with trophozoites from clone L-6, and hybrids were selected by their ability to grow in the presence of emetine. Eight hybrid populations were independently generated. These hybrids showed an intermediate emetine-resistance and rate of phagocytosis. Four of them grew very poorly in semisolid agar and one of them showed a higher virulence than Clone C9, suggesting genetic complementation. Two-dimensional gels of ribosomal proteins showed that clone C9 and L-6 differ in at least one ribosomal protein. The hybrid pattern was different from both parental strains.

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- 392 DETECTION OF E. histolytica IN STOOL SAMPLES BY DNA SPOT HYBRIDIZATION. Acuna-Soto R., Samuelson JC., Biagi F., Wirth DF. Department of Tropical Public Health, Harvard School of Public Health and Department of Pathology Brigham and Women's Hospital, Boston, MA.

We have made a DNA hybridization probe for diagnosis of E. histolytica by blotting rather than by microscopy, which is tedious and difficult. The diagnostic probe recognizes a tandemly repeated, species-specific segment of DNA, which composes about 10% of the E. histolytica genome. Here, parasites were identified by conventional flotation methods and microscopy in 125 stool samples from patients referred to the Laboratorio de Parasitologia in Mexico City. Without diagnosis, the samples were frozen at -20°C and transported to Boston, where they were freeze-thawed x3 to break cysts, in the presence of 500 mM EDTA to inhibit DNase activity in stool. The suspensions were centrifuged at 14,000 rpm and the supernatant spotted onto Gene-Screen Plus and hybridized with radiolabeled (<sup>32</sup>P) probe. All twentyfive samples which were positive for E. histolytica by microscopy were also positive by hybridization (sensitivity 1.0). 94% of the negative samples by microscopy were also negative by hybridization (specificity 0.93). The probe did not significantly cross react with E. coli, E. nana, G. lamblia, Ch. meslini and T. trichuria. The minimum amount of detected cysts was between 10 and 50. This test can be very useful for the detection of carriers of E. histolytica in endemic areas and probably can be used also for the detection of the parasite in the environment.

- 393 MURINE T-CELL CLONES AGAINST ENTAMOEBIA HISTOLYTICA: IN VIVO AND IN VITRO CHARACTERIZATION.

M. Denis\* and K. Chadee. Institute of Parasitology of McGill University, Ste-Anne-de-Bellevue, Québec, Canada H9X 1C0

Eleven T-cell clones were raised from the spleens of BALB/c mice hyperimmunized against a crude soluble extract of Entamoeba histolytica trophozoites. Seven clones were of the Lyt-1<sup>+</sup>, and four the Lyt-23<sup>+</sup> phenotype. All clones proliferated in the presence of E. histolytica antigens but not to a purified protein derivative; five clones proliferated to a crude extract of the E. histolytica-like Laredo amoebae. Ten clones secreted T-cell growth factors in response to E. histolytica antigens. Two clones (Lyt-23<sup>+</sup>) mediated direct-lymphocytotoxicity (73 and 86%) against amoebic trophozoites that was inhibited with rabbit anti-mouse TNF  $\alpha$ . Supernatants of five of the clones (all Lyt-1<sup>+</sup>) activated mouse peritoneal M $\phi$  to kill E. histolytica trophozoites in vitro independent of secreted reactive oxygen intermediates (O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub>). Three of these clones induced IL-1 release by naive M $\phi$ , and all five clones mediated a local DTH reaction in mouse footpad. Our results demonstrate direct lymphocyte cytotoxicity via a cytolytic molecule antigenically related to TNF  $\alpha$  and lymphokine activating M $\phi$  for amoebic killing by a non-oxidative mechanism. (Supported by MRC Canada).

## T: AMEBIASIS AND GIARDIASIS

- 394 DEVELOPMENT OF CELL MEDIATED IMMUNITY IN PEYER'S PATCHES OF GIARDIA MURIS-INFECTED MICE. D.R. Hill, R. Pohl\*. Division of Infectious Diseases, University of Connecticut School of Medicine, Farmington, CT.

Gastrointestinal immune responses are important in the clearance of Giardia. The major site for initiation of these events is the Peyer's patch (PP). We studied, therefore, the development of cell mediated immunity to Giardia muris (GM) in the PP's of mice. Six week old female BALB/c mice were given  $10^3$  GM cysts by esophageal cannula and followed at days 0, 7, 10, 14, 21, 28, 35, 49, and 63. At each time point stool cyst counts were obtained from 2 infected mice and 2 uninfected controls, the mice were sacrificed, and their PP's dissected out. PP cells were concentrated to  $1 \times 10^5$  cells/well, and incubated in triplicate with  $50 \mu\text{g/ml}$  of GM protein or ovalbumin as a control, in a 72 h lymphocyte transformation assay. Optimal cell and antigen concentrations had been determined previously. Non-immune irradiated spleen cells were used as accessory cells. Data is expressed as the stimulation index (SI): (CPM of PP cells + antigen + spleen cells)/(CPM of PP cells + spleen cells). The SI in cells from infected mice + GM antigen began to rise at day 7, reached a peak of 4.8 at day 14, and remained elevated through day 35. This SI was significantly greater than both the SI for control cells from uninfected mice + GM antigen from days 7-35 ( $3.3 \pm 1.4$  (SD) vs.  $1.6 \pm 0.3$ ,  $P < 0.04$ ), and the SI for infected cells + ovalbumin ( $P < 0.04$ ). The SI for control cells did not rise above 2.1, and did not differ from the SI for infected cells + ovalbumin ( $P = 0.63$ ). Peak cyst excretion occurred from days 7-21 and then declined below detectable levels by day 28.

The development of cell mediated immunity in PP's correlated with clearance of GM from mouse intestine. Further elucidation of these events will be useful in understanding the immunobiology of giardiasis.

- 395 ANTIGENIC VARIATION OF GIARDIA LAMBLIA IN HUMAN EXPERIMENTAL INFECTIONS

Nash, T.E., Harrington, D.A., Losonsky, G.A., Levine, M.M., Conrad, J.T. and Merritt, J.W., Jr. Laboratory of Parasitic Diseases, NIAID, Bethesda, Md. and Center for Vaccine Development, University of Maryland, Baltimore, Md.

Surface antigenic variation of Giardia lamblia occurs in vitro and in experimentally infected gerbils. To determine if Giardia surface antigens vary in human infections, 13 and 4 volunteers were inoculated enterally with 50,000 trophozoites of GS/M clones B6 or H7 respectively. B6 possesses a 200kd surface antigen recognized by Mab 3F6 and H7 has a 70kd surface antigen recognized by Mab G10/4. 1/13 of the B6 and 4/4 of the H7 inoculated volunteers became infected. Analysis of Giardia obtained from the intestines on day 21 of 4 H7 infected volunteers and cultures derived from these trophozoites revealed loss of the initial major surface antigen as determined by surface IFA using Mabs, surface radiolabelling, loss of cytotoxicity to Mabs and Western blots. The initial 200kd surface antigen was almost totally absent from the surface of Giardia isolated from the single B6 infected volunteer. Serum surface reactive antibodies as measured by IFA and cytotoxicity to H7 and the day 21 isolates showed high levels of antibody to H7, primarily to the 70kd antigen, but negligible or low levels of late appearing antibodies to day 21 isolates. Day 21 isolates from H7 had some common surface antigens. These studies document antigenic variation of Giardia in human infections and show responses are isolate specific.

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ENCYSTATION-SPECIFIC ANTIGENS OF GIARDIA LAMBLIA.

396 D.S. Reiner\*, H. Douglas and F.D. Gillin. University of California at San Diego Medical Center, San Diego, CA.

We have induced Giardia lamblia to encyst in vitro for the first time and have observed expression of stage-specific cyst antigens which are not detected in cultured trophozoites. Cyst antigens are identified in Western blots using polyspecific rabbit antiserum against cysts purified from human feces. This antiserum reacts with cysts, but not trophozoites, in immunofluorescence, ELISA, and Western blots, while pre-immune serum does not. The optimal condition for in vitro expression of cyst antigens as detected by immunoblotting, was 48 hours' incubation in Diamond's TYI-S-33 medium at pH 7.8 without bovine bile, supplemented with glycocholic, myristic and oleic acids. The major cyst antigens observed were: four discrete high molecular weight bands (68,78,90, 105 kD) and a group of polydisperse low molecular weight bands (29,32,38 kD). The 29-38 kD antigens appear to be under less stringent regulation than the 68-105 kD antigens. The former are observed in encysting cultures as early as 8 hours in the above medium and also under suboptimal encystation conditions. In contrast, the 68-106 kD bands are expressed only after 20 hours under conditions leading to subsequent production of water-resistant cysts. Thus, the encystation antigens are not expressed coordinately. Other studies indicate that they are geographically conserved and that some are expressed on the cyst surface.

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ORGANISATION, SYNTHESIS AND STRUCTURE OF CUTICULAR PROTEINS OF *BRUGIA* Sp.

M.E.Selkirk,\* M.Yazdanbakhsh, M.Blaxter, W Gregory, G.E.Kwan-Lim, E.Cookson, W.Paxton and R.M.Maizels. Imperial College of Science &amp; Technology, London SW7 2AZ.

Surface-labelling techniques have been used to delineate three classes of proteins from the cuticle of *Brugia malayi* and *Brugia pahangi* filarial nematodes which differ in solubility, localisation and pattern of synthesis. The bulk of the cuticular matrix is made up of (minimally) 20 acidic collagenous proteins which are localised in the basal and inner cortical layers. These collagens are relatively small proteins cross-linked by disulphide bonds and are synthesized discontinuously immediately preceding and during the moult. They are encoded by a multi-gene family and each life cycle stage exhibits a characteristic set of constituent collagens. Two non-collagenous proteins of molecular weight 15 Kd and 29 Kd, soluble in aqueous buffer, are also present in the cuticles of adult *Brugia*. Both the former non-glycosylated protein and the latter glycoprotein are synthesized continuously during adult life as higher molecular weight precursors prior to export into the cuticular matrix, and are highly immunogenic in natural infection. The 29 Kd glycoprotein is also found in L<sub>4</sub>, whereas the 15 Kd protein is represented in both L<sub>3</sub> and L<sub>4</sub>. Pulse-chase studies indicate that both proteins are released into culture media; the 15 Kd protein at a high rate. Finally, the epicuticle was also found to be proteinaceous in content, but insoluble in SDS/UREA and 2-mercaptoethanol. This physical property facilitates its purification and antisera has been raised in order to isolate constituent proteins from gene banks. cDNA clones putatively coding for both the 15 Kd and 29 Kd components have been isolated. DNA sequence analysis of the latter clones suggests that the 29 Kd protein has extensive regions of a repeated 5 amino-acid motif. The role of these proteins as possible targets of immunity is being investigated.

398 BIOCHEMICAL AND IMMUNOLOGICAL CHARACTERIZATION OF THE SURFACE ANTIGENS FROM ADULT *DIROFILARIA IMMITIS*.

Alan L. Scott,\* David A. Moraga, M.S. Ibrahim and Wesley K. Tamashiro. Department of Immunology and Infectious Diseases, School of Hygiene and Public Health, The Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205

The ability of the adult forms of filarial parasites to persist long-term in immunologically intact host implies that filariae have evolved mechanisms through which they can evade or modify host defence reactions. Characterization of the surface molecules presented by the adult parasite to the host and the immune reactions against these surface components may afford an insight into the immunobiology of filarial infections. The molecules associated with the surface of adult *Dirofilaria immitis* were identified and characterized employing IODO-GEN-mediated surface labelling, SDS-PAGE and autoradiographic methods. *D. immitis* female and male parasites were found to have a limited number of surface-associated proteins (17.5, 16 and 14.5 kDa) and glycoproteins (49 and 20 kDa) which were readily extracted from parasite homogenates in the absence of detergent. The major surface labelled proteins and glycoproteins were antigenic in rabbits, but appeared to be poorly immunogenic in naturally infected dogs. In addition, a 10 to 6 kDa surface-associated glycolipid was identified which may form a coat on the outside of the parasite and play a role in immune evasion in *D. immitis*. In immuno-precipitation experiments, the glycolipid was not recognized by the antibodies from rabbits immunized with the glycolipid or by antibodies from naturally infected animals. The glycolipid and the 14.5 kDa surface protein were selectively released by the adult parasite during *in vitro* culture.

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- 399 ANALYSIS AND AFFINITY PURIFICATION OF CUTICULAR PROTEINS OF BRUGIA MALAYI AFTER BIOTINYLATION OF INTACT ADULT PARASITES. R.M. Alvarez\* and G.J. Weil. The Jewish Hospital at Washington University Medical Center, St. Louis, MO.

The cuticle of filarial nematodes is a dynamic structure which may be an important target for protective host immune responses. Prior studies have employed radioiodination of intact parasites to demonstrate that the collagenous cuticle of filariids contains relatively few exposed proteins, some of which are stage and/or species-specific. In the present study we have used sulfo-NHS-biotin to label and affinity-purify cuticular proteins of living adult Brugia malayi. Results obtained by this method were compared with the widely used Iodo-gen method of surface iodination by SDS-PAGE analysis of detergent-solubilized worms and by ultrastructural analysis. Both labeling methods produced very similar SDS-PAGE patterns with labeled major protein doublets at 70 and 100 kDa, a major band at 25 kDa, and minor bands between 60-200 kDa. Ultrastructural analysis showed that both methods labeled components throughout all levels of the parasite cuticle; underlying somatic tissues were not labeled. Advantages of biotinylation are stability of the label (SDS-PAGE patterns of solubilized proteins were unchanged after 12 months at 4°C) and the ability to affinity-purify labeled cuticular components on avidin-agarose for use in production of anticuticular antibodies. Thus, biotinylation of intact filarial parasites is a simple method which can be used for analysis and isolation of cuticular proteins.

- 400 QUALITATIVE CHARACTERIZATION OF ANTIBODY RESPONSES OF JIRDS TO BRUGIA PAHANGI INFECTION. R.G. Farrar\*, T.R. Klei, C. McVay and S.U. Coleman. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803

Qualitative antibody responses of single and multiply-infected jirds with infections of varying duration (19-188 days post infection, DPI) to whole and sectioned life cycle stages of the parasite and to a soluble somatic extract (SE) of adult parasites were characterized by indirect fluorescent antibody (IFA) and Western blot analysis. In IFA, maximal, sustained antibody responses were observed to reproductive organs in the adults and in somatic structures of L<sub>3</sub>, L<sub>4</sub> and microfilariae by 54 DPI. Surfaces of washed eggs released from ruptured uteri were reactive with sera by 60 DPI whereas eggs released by females *in vitro* were not. Western blot analysis of SE identified 42 protein bands ranging in molecular weight from 12 to 160 KD. Recognition of proteins with molecular weights 37, 21, 17 and 15 KD appeared to correlate with the time course of infection. The 37 KD protein was recognized at the onset of patency. Recognition of the 21 KD protein paralleled the rise in microfilaremia in the jird. Recognition of the 17 KD protein paralleled the time course of granulomatous responsiveness to antigen whereas the 15 KD protein was recognized only briefly during the period of maximum down regulation. Antibody recognition of B. pahangi antigens in the jird appears to be independent of; ELISA antibody titers to crude antigen, severity of lymphatic lesions, levels of microfilaremia, numbers of L<sub>3</sub> inoculated or adults recovered. In this model a clear relationship between antibody and the pathogenesis of lymphatic lesions was not seen.

- 401 MOLECULAR MIMICRY OF A SURFACE EPITOPE OF BRUGIA MALAYI INFECTIVE LARVAE BY ANTI-IDIOTYPIC ANTIBODIES. Clotilde K.S. Carlow,\* P. Busto and Mario Philipp. Molecular Parasitology Group, New England Biolabs, Beverly, MA.

We have previously reported the production of a mouse monoclonal IgM antibody (AB 1) against a surface epitope of Brugia malayi infective larvae which is species- and stage-specific. AB 1 was unsuitable for antigen purification by affinity chromatography. In the present study anti-idiotypic antibodies (AB 2) were raised in rabbits which strongly inhibit the binding of AB 1 to its target epitope. In contrast, AB 2 did not inhibit the binding of an IgM monoclonal antibody specific for a surface epitope of Dirofilaria immitis infective larvae. Immunization of BALB/c mice with affinity purified AB 2 elicited anti-filarial antibodies (AB 3) reacting with the surface of living B. malayi infective larvae and bearing the same species-specificity as AB 1. This approach is useful to investigate the protective capacity of carbohydrate epitopes, of which there are many amongst surface and secreted filarial antigens, and also in the study of otherwise not easily purified epitopes of any composition.

- 402 ISOLATION AND PARTIAL CHARACTERISATION OF RECOMBINANT ANTIGENS FROM A GENOMIC LIBRARY OF WUCHERERIA BANCROFTI. N. Raghavan\*, C.V. Maina, L.A. McReynolds, and T.B. Nutman. Laboratory of Parasitic Diseases, NIH, Bethesda, MD and New England Biolabs, Beverly, MA.

In the study human lymphatic filariasis, progress has been hampered by: 1) the lack of defined parasite antigens; 2) the broad immunological cross-reactivity seen among the eight filarial species of humans; and 3) the dearth of abundant parasite material. To define and generate filarial proteins that are important in inducing parasite-specific immune responses in the human host and to understand, at a molecular level, the differences among related filarial species, a genomic DNA library from Wuchereria bancrofti (Wb) microfilariae isolated from patients in Madras, India, was constructed in  $\lambda$  gt11 after EcoR1 digestion. This library (initial titre  $1.1 \times 10^6$  pfu/ml) was amplified and 29 positive clones obtained from  $3 \times 10^5$  recombinants screened with pooled sera from bancroftian filariasis patients. Two of the 29 clones ( $\lambda$ WbN-1 and  $\lambda$ WbN-6) showed inserts of approximately 2.5kb when probed with nick translated total Wb DNA and yielded fusion proteins of 131kD and 127 kD by immunoblotting.

In Southern blot analysis  $\lambda$ WbN-1 recognizes sequences in EcoR1- digested Wb DNA (2.5 kb) and B. malayi DNA (2.2 kb) but not in DNA from any of the 5 other filarial (B. pahangi, D. immitis, Q. volvulus, A. vitiae, L. carinii) or 2 non-filarial (C. elegans, human) DNAs tested. Lysogens of this clone and  $\lambda$ WbN-6, when screened by dot blot ELISA, showed strong reactivity with sera from Wb-infected individuals but did not react with normal human sera.  $\lambda$ WbN-1 and  $\lambda$ WbN-6 were also subcloned into the vector pCG806R $\alpha$  (containing the MalE binding protein), and their restriction maps and partial sequences have been obtained.

These clones and other recombinants obtained from this first Wb library should provide further insights into the biology of Wb and the immune responses it elicits.

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ANTIGEN SHEDDING FROM THE SURFACE OF  
DIROFILARIA IMMITIS INFECTIVE LARVAE

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The turnover of surface peptides of developing *Dirofilaria immitis* infective stage larvae (L3) was assessed quantitatively and qualitatively. L3s were surface labelled using the <sup>125</sup>I-Iodo-Gen method and maintained in *in vitro* culture for 6 days. During this period, larval survival, larval development and rate of surface peptide release were monitored. About 10-20% of radiolabelled peptides were lost from developing larvae daily until the time of molting (day 4). After molting to the fourth larval stage (L4), all of the radioactivity was found associated with the exuviae and no radioactivity was associated with the L4, indicating that radiolabelling was restricted to the outer surface of L3s. The loss of surface labelled peptides from developing larvae corresponded with an increase in the amount of radioactivity found in the culture medium. Analysis of surface-associated and shed material by SDS-PAGE and autoradiography showed that the peptides released by L3s into the medium had the same apparent molecular weights, 35 and 11 kDa, as the major labelled peptides found on the surface. Developing larvae obtained from culture after 1, 2, 4, and 6 days exhibited reduced ability to bind anti-L3 antibodies from a rabbit immunized with living *D. immitis* L3s. The 35 kDa molecule from surface-labelled L3 extracts and from culture medium were immunoprecipitated by rabbit anti-L3 antibodies. In addition, sera from naturally infected dogs immunoprecipitated the 35 kDa from surface labelled larval extracts. The 11 kDa peptide was not recognized by either sera. These results demonstrate that *D. immitis* infective larvae undergo a relatively high rate of surface protein shedding accompanied by a reduction in surface antigenicity during development from L3 to L4 *in vitro*. The shedding of surface peptides and the reduced surface antigenicity may represent a stage-dependent mechanism of immune avoidance.

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PURIFICATION AND BIOCHEMICAL AND IMMUNOLOGIC CHARACTERIZATION OF A  
25KD GLYCOPROTEIN FROM THE SURFACE OF DIROFILARIA IMMITIS FOURTH  
STAGE LARVAE

T.B.Davis\* and M. Philipp. Molecular Parasitology Group, New England Biolabs, Beverly, MA.

Surface antigens of the larval stages of filarial parasites are considered prime targets for immunoprophylaxis. While surface antigens of infective stage larvae of several filarial parasites have been examined in detail, limited data are available about the surface of the 4th stage larva, although this stage may be a better target for immunoprophylaxis because it is present in the host for a longer period of time. The principal antigens identified on 4th stage larvae of *Dirofilaria immitis* by surface iodination have electrophoretic mobilities of 150, 52, and 25 kD by SDS-PAGE. The 25 kD molecule does not appear to be expressed on the surfaces of third stage larvae or adult parasites, however it can be found in extracts of adults. Purification of this component from adult *D. immitis* by 2-D PAGE has allowed defined biochemical and immunologic analysis. The 25 kD molecule is antigenic in dogs vaccinated with irradiated third stage larva of *D. immitis*, and retains its antigenicity after enzymatic deglycosylation. Monospecific sera against this antigen generated in rabbits and mice are currently being used to characterize clones from *Dirofilaria immitis* expression libraries.

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405 A 16 kD SURFACE ANTIGEN OF BRUGIA MALAYI EXPRESSED BY 4TH STAGE LARVAE, ADULT WORMS AND BY POST - PARASITIC 3RD STAGE LARVAE.  
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Antigens expressed on the surface of filarial nematodes are considered *prima facie* vaccine candidates since they may be the targets of lethal immune effector mechanisms. We have been investigating the surface antigens of *Brugia malayi* larvae. Due to the paucity of larval material the identification and characterization of such antigens would be greatly simplified were the larval antigens to be co-expressed by the more abundant adult worms.

Using protein iodination techniques we have determined that of the three detergent soluble surface antigens expressed on the surface of adult worms of *B. malayi* (16, 20 and 29 kD) one, the 16 kD component, is also expressed by post - parasitic third stage and fourth stage larvae. The 16 kD band of all stages shared antigenic determinants and co-electrophoresed to the same pI on 2-D gels. Metabolic labelling of adult worms and subsequent co-two - dimensional electrophoresis with extracts of surface radio-iodinated worms demonstrated that the major 16 kD component is actively produced by adult males and is therefore likely to be represented in our cDNA expression libraries derived from their mRNA. Interestingly, expression of the 16 kD molecule is not apparent in third stage larvae prior to infection of the vertebrate host. Therefore this developmentally regulated surface molecule may be functionally important in the vertebrate portion of the parasite's life cycle.

406 A HYDROPHOBIC GLYCOSYLATED COMPONENT ON THE SURFACE OF ONCHOCERCA LIENALIS MICROFILARIAE. D.E.Hill\*, J.J.Donnelly, M.Khatami, J.B.Lok, and J.H.Rockey. Univ. of Pa., Phila., Pa.

We have studied the surface components of microfilariae (mfs) of *Onchocerca lienalis*. Previously, we demonstrated that IgG antibody from subconjunctivally immunized guinea pigs did not bind to the surface of intact mfs, but did bind to internal antigens, and that 5mM DNFB did not label primary amino groups on the surface of intact mfs. We extracted the surfaces of intact mfs with 5% phenol in PBS. Phenol extracted and control mfs were incubated in 5mM DNFB, then in MOPC-315 (anti-DNP) and FITC-anti-mouse antibody or were incubated in immune serum from subconjunctivally immunized guinea pigs, then in FITC-anti-guinea pig antibody. Both groups of extracted mfs fluoresced, while unextracted control mfs did not. Western blot analysis of intact mf 'surface' labelled with NHS-SS-biotin, which binds covalently to 1' amino groups, revealed a band with a MW of about 20kD. Western blot analysis of surface extracted, DNFB labelled mfs revealed a band with an identical MW. While no evidence of DNP labelling was seen on unextracted mfs. The phenol extracted surface material was applied to silica gel G TLC plates and chromatographed in 2 dimensions, then sprayed with 1% orcinol/10% sulfuric acid revealing 1 or 2 glycosylated lipid species which comigrated with glycolipid standards. Phospholipids and cholesterol were not detected. These results indicate the presence of an organic solvent extractable, glycolipid-like coat on the surface of *O. lienalis* mfs, which may prevent binding of ligands to the surface of mfs and protect mfs from host defense mechanisms.

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407 DIFFERENTIAL RECOGNITION OF A MICROFILARIA-SPECIFIC ANTIGEN OF BRUGIA MALAYI.

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IgG antibodies to surface antigens of microfilariae participate in the control of parasitemia due to lymphatic filariasis. To identify worm antigens that elicit such antibodies, we compared antigen recognition patterns of persons who remained amicrofilaremic after 3-6 years of exposure to *B. malayi* with those of patients who developed patent filariasis during the same period. All sera identified multiple antigens on western blots of microfilarial extracts but only sera from amicrofilaremic donors reacted with 70/75 kD parasite molecules. A proportion of these sera contained IgG antibodies that compete with the binding of a monoclonal antibody (Mab MF1) to its epitope on a microfilarial stage-specific antigen; this Mab reduces microfilaremia in infected jirds. These results suggest that the epitope recognized by Mab MF1 might be the target of a protective IgG response in patients with brugian filariasis.

408 STUDIES ON ANTI-MICROFILARIAL IMMUNITY USING *DIROFILARIA IMMITIS* IN LEWIS RATS.

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Studies were conducted to determine the effect of vaccination on rates of microfilarial clearance using *Dirofilaria immitis* in male Lewis rats. Animals were immunized with either of two antigen preparations emulsified in Freund's adjuvant: (1) whole, dead microfilariae or (2) a PBS extract of microfilariae. The immunized animals, as well as untreated and adjuvant controls, were challenged intravenously with  $4 \times 10^5$  viable microfilariae. Vaccination significantly enhanced the ability of Lewis rats to clear parasites from the circulation. The duration of microfilaremia in the four groups of rats were 15.5, 17.7, 36.3 and >70d for rats vaccinated with "whole mf", a PBS extract of microfilariae, adjuvant alone and the untreated group, respectively. Analysis of the anti-microfilarial IgG response by ELISA and Western blots demonstrated that immunization induced significant amounts of antibody against high molecular weight peptides, in particular, a peptide located at 105 kDa. Antibody levels in both groups of immunized animals continued to rise following challenge, reaching peak levels of 78-80  $\mu\text{g/ml}$  on the day of microfilarial clearance. Decreasing microfilaremia following challenge was associated with an enhanced recognition of low molecular weight peptides.

WORKSHOP: CELL-MEDIATED IMMUNITY TO ASEXUAL MALARIA PARASITES

- 409 INTRODUCTION. L.H. Miller. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD; W.P. Weidanz, Hahnemann University School of Medicine, Philadelphia, PA; J.H.L. Playfair, Middlesex Hospital Medical School, London, ENGLAND; and I.A. Clark, Australian National University, Canberra, AUSTRALIA.
- 410 K.N. Brown. National Institute of Medical Research, Mill Hill, UNITED KINGDOM.
- 411 S. Kumar. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD.
- 412 R. Mogil. University of Alberta, Edmonton, Alberta, CANADA.
- 413 D.J. Wyler. Tufts University School of Medicine, Boston, MA.

**INTRODUCTION:** Cell-mediated immunity to the asexual erythrocytic parasite has been established in primate malarias by the Taliaferros and in rodent malarias by Clark and Allison and by Weidanz and his colleagues. The character of this immunity is as follows. The effector mechanism is not antigen specific and leads to intraerythrocytic death of asexual parasites. The mechanism is, in general, dependent on the spleen; immune animals lose some or all of the immunity following splenectomy. The induction and effector mechanisms are antibody independent but T cell dependent. The immunity can be transferred to nonimmune mice by CD4+ T cell lines and CD4+ T cell clone lines in Plasmodium chabaudi adami, a rodent malaria.

**FORMAT:** The round table discussion will be the format for exploring the validity of these ideas and their extension to human malaria. The meeting will begin with the exposition of the ideas in the literature and from presentations in an earlier meeting. Speakers will then give 15 min. presentations of views that will test, question or extend the concepts in this area. Members of the panel and audience will then discuss and debate the ideas presented by the speakers. We will then discuss the relevance of cell-mediated immunity in human malarias, especially P. falciparum. The goal of the round table is to define the important issues that need further research in cell-mediated immunity in malaria.

ANNUAL SCIENTIFIC MEETING OF THE AMERICAN COMMITTEE OF MEDICAL ENTOMOLOGY (ACME)

RECENT APPROACHES TO THE STUDY OF SYSTEMATICS AND EVOLUTION OF  
ARTHROPOD VECTORS AND ARTHROPOD-TRANSMITTED PATHOGENS

- 414 INTRODUCTION AND SCOPE. B.F. Eldridge. Department of Entomology,  
University of California, Davis, CA.
- 415 CURRENT TRENDS IN STUDIES OF THE GENETIC STRUCTURE OF VECTOR  
POPULATIONS. L.E. Munstermann. Vector Biology Laboratory, University  
of Notre Dame, IN.
- 416 RECENT DEVELOPMENTS IN GENETICS AND SYSTEMATICS OF TICKS. J.H.  
Oliver, Jr. Department of Biology, Georgia Southern College,  
Statesboro, GA.
- 417 THE USE OF RIBOSOMAL AND MITOCHONDRIAL DNA TO STUDY POPULATION AND  
SPECIES RELATIONSHIPS IN ANOPHELINES. F.H. Collins. Centers for  
Disease Control, Atlanta, GA.
- 418 A GENETIC APPROACH TO THE STUDY OF TICK TRANSMITTED ARBOVIRUSES  
(ORBIVIRUSES). P.A. Nuttall. National Environment Research Council,  
Institute of Virology, Oxford, UNITED KINGDOM.
- 419 IDENTIFICATION OF SPECIES OF PLASMODIUM. R.A. Wirtz. Department of  
Entomology, Walter Reed Army Institute of Research, Washington, DC.
- 420 MOLECULAR IDENTIFICATION AND CLASSIFICATION OF SPECIES OF LEISHMANIA.  
D. McMahon-Pratt. Yale University School of Medicine, New Haven, CT.

DISCUSSION AND QUESTIONS.

## V: RETROVIRAL INFECTIONS AND EPIDEMIOLOGY

- 421 HUMAN EOSINOPHILS CAN EXPRESS CD4 AND HLA-DR, AND BIND HIV GP120.  
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Beth Israel Hospital, Boston, Massachusetts.

Eosinophils are tissue-dwelling (99%) leukocytes associated with hypersensitivity states and helminthic infections. In central Africa helminths are endemic and heterosexual transmission of AIDS is more common than in most developed nations. We asked whether eosinophils could contribute to this epidemiologic difference and found that under certain conditions eosinophils can express CD4 and HLA-DR as well as bind the human immunodeficiency virus envelope glycoprotein (HIV gp120). Eosinophils from 9 donors (5 with eosinophilia of 11-74%) were analyzed by flow cytometry before and during culture (up to 10 days) with granulocyte-macrophage colony stimulating factor and murine fibroblasts. CD4 was expressed primarily in donors with eosinophilia, particularly during culture (peak of 12.6-92.1% CD4+ cells). HLA-DR was expressed on all 9 donors during culture (peak of 24.1-97.4% HLA-DR+ cells), and 1/9 prior to culture. Biosynthetic labelling with (35S)-methionine and immunocytochemical staining corroborated cytofluorography data. Binding of partially purified HIV gp120 to CD4+ eosinophils was demonstrated cytofluorographically by competitive inhibition of OKT4A (HIV-binding epitope), but not OKT4 monoclonal antibody binding.

We have found that eosinophils can express CD4 and HLA-DR, that expression is enhanced during culture, and that CD4+ eosinophils can bind HIV gp120. We conclude that eosinophils may contribute to the processing of foreign antigen via HLA-DR expression and to the pathogenesis of HIV/AIDS via CD4 expression, particularly in eosinophilic states.

- 422' HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION IN EGYPTIANS IN CAIRO.  
F.S. Galal\*, M. Kamal\*, M. Haphez, Y. Safwat, N. Bassiouni, Z. Farid,  
and J.N. Woody. Abbassia Fever Hospital (AFH), U.S. Naval Medical  
Research Unit No.3 (NAMRU-3), and the Ministry of Health, Cairo, Egypt.

Although HIV infection is endemic in many Subsaharan African countries, recent serosurveys have shown that it has not yet spread into Egypt. This is the first clinical description of this infection in Egyptians. During 1987-88, 12 Egyptian patients, 8 men and 4 women aged 1 to 42 years were referred to the AFH for investigation of suspected HIV infection. In every case evidence of infection was diagnosed by the enzyme-linked immunosorbent assay (ELISA) and confirmed by Western Blot. All 12 patients were in the high risk group. Six had received blood transfusion outside Egypt. One was the wife and one the daughter of AIDS patients. One was an intravenous drug abuser and one gave a history of sexual contact with prostitutes; both worked in Italy. The remaining 2 patients were homosexuals; one worked in the Gulf and one in West Germany. Four of the 12 patients presented with AIDS and were seriously ill with fever, diarrhea, marked weight loss, and generalized lymphadenopathy (2 died within 2 months of admission to hospital). A girl aged 6 years presented with prolonged fever with Salmonella bacteremia, and a 21 year-old haemophilic male presented with generalized lymphadenopathy. The 6 remaining HIV infected patients were asymptomatic and are under observation.  
(Supported by NMRDC, Bethesda, M.D. Work Unit No. 3M463105DH29.AA.002).

## V: RETROVIRAL INFECTIONS AND EPIDEMIOLOGY

### SEROLOGIC EVIDENCE FOR HIV-2 IN EAST AFRICA

423 N.T. Constantine\*, D.M. Watts, R.L. Daise, and J.N. Woody. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt

Human immunodeficiency virus type 2 (HIV-2) is endemic in West Africa and was recently reported from Europe, South America, and North America. Our objective was to assess serologic reactivity for HIV-2 in HIV-1 reactive sera from four East-African countries. Fifty-three sera were assayed, 27 of which were HIV-1 Western Blot (DuPont) confirmed (reactivities to at least p24 and gp41) and also positive by the Virgo IFA (>160). Twenty-six sera were HIV-1 ELISA positive only. All sera were screened for HIV-2 antibodies by a micro-ELISA (Genetic Systems). Sera exhibiting  $\Delta OD$  readings  $>.300$  repeatedly on three occasions, were further characterized by Western Blot (Pasteur) analysis for HIV-2 confirmation. Among the 26 HIV-1 ELISA positive only sera, none met the criteria for HIV-2 positivity by the HIV-2 ELISA. In contrast, 7 of the 27 (26%) confirmed HIV-1 positive samples reacted unequivocally and repeatedly positive by the ELISA ( $\Delta OD$  .35-1.9 for HIV-2 antibodies. When subjected to Western Blot analysis for HIV-2, 4 of these 7 sera reacted to gp105, thus fulfilling the manufacturer's criteria for HIV-2 antibody positivity. Careful examination of the reactivities of these 4 sera by HIV-1 and HIV-2 Western Blot analysis revealed that two of the sera were broadly reactive by both blots. The two remaining sera exhibited very weak reactivities to only the HIV-1 envelope proteins gp120-160. One of these exhibited a weak banding pattern between gp120 and 160, suggesting a reaction with gp140. In conclusion, four human sera from East Africa were identified as reactive by both HIV-1 and HIV-2 Western Blots; two of these sera produced profiles more indicative, and highly representative of HIV-2 exposure. (Supported by NMRDC, Bethesda, MD Work Unit No. 3M463105.H29.AA.335.

### HUMAN IMMUNODEFICINECY VIRUS IN SUDANESE PROSTITUTES

424 M.C. McCARTHY\*, N.T. CONSTANTINE, R.L. DAISE, EL-SADDIG MAGOUB  
U.S. NAVAL MEDICAL RESEARCH UNIT No.3, CAIRO, EGYPT, MINISTRY OF  
HEALTH, KHARTOUM, SUDAN.

We enrolled 203 prostitutes, 102 long-distance truck drivers and 73 sailors into an epidemiologic study to determine the prevalence and incidence of HIV-1 and HIV-2 among prostitutes and their transient customers.

Epidemiologic questionnaires revealed that prostitutes averaged 46 sexual partners per month, 58% had given birth to an average of 1.7 children and 88% reported a history of travel within Sudan. Among sailors, 50% were married and 47% had patronized prostitutes during the past seven years, 94% of whom reported a history of STD. Among truck drivers, 41% were married and 75% admitted to sexual relations with prostitutes during the past seven years, 65% of whom reported a history of STD.

All initial sera were negative for antibody to HIV-1 or HIV-2. Forty-six sera obtained from prostitutes at six-month follow-up were negative for HIV-1 antibody and antigen, as well as HIV-2 antibody.

In conclusion, this study demonstrates that the prevalence and incidence of HIV-1 and HIV-2 infections are low in Port Sudan, and suggests that they have not yet been introduced into this high-risk population. Additionally, it illustrates that these groups represent mobile reservoirs of STD, and may herald dissemination of HIV in Sudan.

(Supported by NMRDC Bethesda, MD., Work Unit No. 3M463105.H29.AA.335)

## V: RETROVIRAL INFECTIONS AND EPIDEMIOLOGY

- 425 HIGH PREVALENCE RATES OF HUMAN T-LYMPHOTROPIC VIRUS TYPE I (HTLV-I) INFECTION IN ISOLATED POPULATIONS OF THE WESTERN PACIFIC WITHOUT JAPANESE OR AFRICAN CONTACT. R.M. Garruto\*, P. Slover, C. Mora, R. Yanagihara, D.M. Asher, P. Rodgers-Johnson and D.C. Gajdusek. National Institutes of Health, Bethesda, MD.

We report zero or very low prevalence rates of antibodies against HTLV-I in Guamanians and Carolinians, despite more than 30 years of intense contact with the Japanese, and high rates in the remote indigenous populations of the Solomon, Banks and Torres Islands and in some primitive, little-visited populations of New Guinea, which had no contact with Japanese or Africans and little contact with Caucasians prior to our bleedings. 1603 sera, collected between 1962 and 1976, were tested by ELISA for IgG antibodies to HTLV-I. Selected sera were also tested by Western immunoblot. Moderate to very high prevalence rates of HTLV-I infection were found in 4 populations from the Solomon Islands: Santa Cruz (9%), Reef Island (11%), Vanikoro (14%), Bellona (21%); in 6 populations from the Banks and Torres Islands: Vanua Lava (21%), Mera Lava (22%), Gaua (38%), Merig (41%), Hiu (47%), Loh (48%); and in 6 New Guinean populations: Touri (13%), Esep (26%), Arebunkara (27%), Obeimi (35%), Waragu (50%). By contrast, rates were low or nonexistent in 4 populations from the Mariana and Caroline Islands of Guam (1%), Woleai (2%), Fais (1%), Satawal (0%) and Truk (0%). Infection rates were similar among males and females, and acquisition of HTLV-I antibodies increased with age. By Western immunoblot, positive sera showed strong reactions with gag-encoded proteins and weak reactivities with env-encoded proteins. Our data indicate that HTLV-I infection has been widespread in the southwestern Pacific for over 25 years in populations with minimal outside contact, while some populations which had extensive Japanese contact now have no infection with HTLV-I.

- 426 THE PREVALENCE OF HUMAN IMMUNODEFICIENCY VIRUS AND HEPATITIS B VIRUS INFECTION IN HIGH RISK GROUPS IN CAIRO.  
S. Bassily\*, N. Constantine, J.N. Woody and F. Sheiba.  
U.S. Naval Medical Research Unit No.3 and Ministry of Health, Cairo, Egypt.

The human immunodeficiency virus (HIV) infection is now spreading in many parts of the world. The epidemiology of HIV infection has many parallels with that of Hepatitis B Virus (HBV) infection. While HIV infection is only recently reported from Egypt, HBV infection is endemic. This report summarizes the seroepidemiologic survey for HIV1, HIV2 and HBV markers in three high risk groups; patients with sexually transmitted diseases, STD (293), intravenous drug abusers (235) and prostitutes (257). The Abbott recombinant EIA was used for detection of anti-HIV1, the micro EIA (Genetic systems) for anti-HIV2, and repeatedly reactive sera were confirmed by Western blot technique (DuPont). Commercial Abbott EIA kits were used for the detection of HBV markers (HBsAg, anti-HBs and anti-HBc). Two of the drug addicts (0.85%) were positive for anti-HIV1, one of them confirmed. Two of STD patients (0.68%) were also EIA positive for anti-HIV1 but with a weak Western blot reaction. All the prostitutes were negative. By comparison over 10,000 sera, collected from various non high risk groups from Egypt were negative for HIV1. There were no positive anti-HIV2 individuals. The prevalence rate of HBsAg was 6.2% in the drug addict group, 4.7% in the prostitutes and 2.7% in patients with STD. Anti-HBs and anti-HBc prevalence rates ranged from 30-50% in the 3 groups. Other risk factors correlating with these results will be discussed.

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V: RETROVIRAL INFECTIONS AND EPIDEMIOLOGY

- 427      EPIDEMIOLOGY OF HTLV-I INFECTION IN REMOTE COASTAL POPULATIONS ON  
         THE PHILIPPINE ISLAND OF PALAWAN  
         C.G. Hayes,\* C.R. Manaloto, R.B. Oberst and L.W. Laughlin. United  
         States Naval Medical Research Unit No. 2, Manila, Republic of the  
Philippines.

The Human T-cell Leukemia Virus (HTLV-1) has been associated with adult T-cell leukemia and, more recently, with tropical spastic paraparesis, a chronic neurological disease. We have been conducting serological surveys to determine if this virus is present in the Philippines. Several communities on the west coast of Palawan, an area highly endemic for malaria, were included in the survey, and 24.6% of the 1,748 residents sampled were shown to have HTLV-I antibody by ELISA and Western Blot. The positivity rate increased with age up to about 8 years and then remained fairly constant. Overall, males had a significantly higher positivity rate than females. Among the 6 communities in which at least 100 people were sampled the positivity rate varied from 8.5% to 34.1%, and even communities less than 5 km apart had significantly different rates that could not be explained by age. Results of a case-control study to identify risk factors will be presented.

- NEUROBEHAVIORAL EFFECTS OF TOXOCARIASIS  
428      T.M. Bailey,\* P. Succop, P.M. Schantz, R.L. Bornschein, K.N. Dietrich,  
         J.K. Stehr-Green. Centers for Disease Control, Atlanta, GA and  
Cincinnati Lead Study, University of Cincinnati Medical Center, Cincinnati, OH.

Infections of humans with Toxocara spp. results in extensive migration of the larval nematodes in tissues. Zoonotic human toxocariasis is common in children. Five to 7% of children in the United States have serologic evidence of infection with serologic prevalence approaching 30% among black children of lower socioeconomic status. A proportion of infected children develop systemic or ocular symptoms of disease. While the majority of infected children show no apparent symptoms of disease, recent studies have associated certain neurobehavioral deficits with Toxocara infection. As pica is a risk factor for both Toxocara infection and lead ingestion, it has not been possible to distinguish the neurobehavioral effects of Toxocara infection from those due to lead ingestion. A prospective study of chronic low-level lead exposure and its effects of childhood neurobiological and psychological development was begun in Cincinnati in 1980. The study protocol examines children from birth through 5 years of age for neurobehavioral development, home environment and laboratory parameters, including serum lead determinations and antibodies to Toxocara spp. Preliminary results on 282 children have identified seventeen children with antibodies to Toxocara spp. that first begin to appear between 2 to 2 1/2 years of age (3.3% prevalence) with peak age specific prevalence being reached between 4 1/2 to 5 years of age (20.6% prevalence) while increased serum lead levels are first detected at 6 months of age. The temporal relationship between Toxocara antibody seroconversion, serum lead levels and neurobehavioral morbidity are discussed.

## V: RETROVIRAL INFECTIONS AND EPIDEMIOLOGY

- 429 GUINEA WORM PILOT CONTROL PROJECTS IN TWO PAKISTANI VILLAGES  
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B.L. Cline, Global 2000, Inc. and Centers for Disease Control,  
Atlanta, and National Institute of Health, Islamabad.

Two villages (Aghzar Khel, NWFP, and Chachi, Sind Province), each distinct ecological/epidemiological zones of dracunculiasis endemicity in Pakistan, were selected to collect descriptive information relevant to control, and to implement and evaluate integrated interventions. This operational knowledge and experience was considered essential to permit rational planning of the national eradication program. Initial demographic and behavioral surveys undertaken in both villages revealed similarities and differences between the two populations which had important operational implications. For example, in Aghzar Khel virtually everyone routinely filtered drinking water, while in Chachi 72% of women and only 14% of men did so. Literacy rates in Chachi were extremely low (4%). In much of the northern part of the endemic zone, represented by Aghzar Khel, transmission of dracunculiasis was found to occur in enclosed rainwater cisterns maintained by households as sources of drinking water. In the affected southern areas, represented by Chachi, transmission occurs mainly via drinking water in large, seasonally-filled depressions in the desert (tarais) which are communally maintained and used. Following the initial surveys, health education measures were introduced in both pilot villages, encouraging use of monofilament filters to eliminate the infected copepods from drinking water, and chemical treatment of drinking water sources with temephos. Compliance with temephos treatment and usage and wear of filters have been assessed longitudinally. This paper presents detailed results and shows how knowledge gained from the pilot control villages is being applied in the national eradication program.

- 430 PAKISTAN: NATIONWIDE SEARCH FOR GUINEA WORM DISEASE (GWD)  
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M. Burney, B.L. Cline, National Institute of Health, Islamabad,  
Global 2000, Inc., and Centers for Disease Control, Atlanta.

As the first step of a Guinea worm eradication programme in Pakistan and in view of widespread passive reporting of GWD in the country, we undertook a nationwide search of all (some 48,000) villages in Pakistan between April and June 1987. The main objective was to identify (for subsequent control) all villages with endemic or recent (within 2 years) GWD (<10 cases or  $\geq 10$  cases). Malaria, EPI and other health workers, school teachers, and local government officials conducted the initial search, assisted in case recognition by color photographs of an emerging Guinea worm. A total of 401 villages were provisionally identified as having endemic GWD, 252 in Sind Province, 79 in North West Frontier Province and 70 in Punjab Province. Only 77 of these 401 villages were found to have  $\geq 10$  cases. A follow-up validation survey conducted in a sample of 102 villages in July/August confirmed a level of accuracy of about 83% in the national search with 12% "false positive" and 5% "false negative" search results. In October 1987 at the end of the season of worm emergence, a special case counting effort was conducted in conjunction with a broader primary health care survey. Using a questionnaire in house-to-house interviews, all 77 villages identified as having  $\geq 10$  cases in the previous active search were visited, as well as a 10% sample of villages with <10 cases. The case counting survey found a gratifyingly low number of cases of dracunculiasis: 171 in NWFP, 248 in Punjab Province, and 447 in Sind Province. The total population at risk of dracunculiasis in the endemic villages of these three provinces is 361,000. Intervention is now underway with a zero case target date of 1990.

## V: RETROVIRAL INFECTIONS AND EPIDEMIOLOGY

- 431 DRACUNCULIASIS ERADICATION IN NIGERIA:  
PROSPECTS AND CHALLENGES.  
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Ilorin, Ilorin Nigeria.

Dracunculiasis is endemic in virtually all the 21 Nigerian States. It is estimated that about 2.5 million cases of the disease occur annually while about a third of the country's estimated population of over 100 million people is at risk. Qualitative and quantitative research findings have documented the adverse impact of the disease on health, agriculture, education, child survival and socio-economic conditions. The activities of local researchers and mass media are rapidly promoting better public awareness about the disease problem, its mode of transmission and its prevention. Results of intervention programs in Kwara and Imo states already provide convincing local proof that guinea worm is highly vulnerable to control measures, and that its elimination is achievable, yielding dramatic socio-economic and other benefits. Five Nigerian states (Anambra, Kwara, Ondo, Ogun, Oyo) have established state task forces for guinea worm eradication, and a national guinea worm task force was also established recently. These domestic initiatives, and the increasing external support of international agencies (e.g. Global 2000, UNICEF, Japan) constitute the beginning of a concerted campaign to eliminate dracunculiasis from Nigeria by 1995. A national state by state search for cases of guinea worm disease will be conducted beginning this summer, followed by the Second National Conference on Dracunculiasis in Nigeria. This paper will summarize the background, challenges, prospects and significance of this program.

- 432 ASSOCIATION OF HEPATITIS E VIRUS (HEV) WITH AN EPIDEMIC OF ENTERICALLY TRANSMITTED NON-A, NON-B HEPATITIS (ENANBH) IN PAKISTAN: DETECTION OF ANTI-HEV IN SERUM AND HEV IN FECES.  
J Ticehurst<sup>1\*</sup>, TJ Popkin<sup>2</sup>, JP Bryan<sup>3</sup>, BL Innis<sup>4</sup>, JF Duncan<sup>5</sup>, A Ahmed<sup>6</sup>, AZ Kapikian<sup>2</sup>, M Iqbal<sup>8</sup>, LJ Legters<sup>3</sup>, and RH Purcell<sup>2</sup>; <sup>1</sup>Walter Reed Army Inst Research, Washington DC; <sup>2</sup>Natl Inst Allergy & Infectious Diseases and <sup>3</sup>Uniformed Services Univ Health Sciences, Bethesda MD; <sup>4</sup>Armed Forces Research Inst Medical Sciences, Bangkok, Thailand; <sup>5</sup>Pakistan-US Lab Seroepidemiology, Army Medical College, Rawalpindi, Pakistan.

Cross-serologic analyses by immune electron microscopy (IEM)<sup>1,2</sup> have defined a single serotype of HEV, a proposed designation for the etiologic agent of ENANBH<sup>3</sup>. In 1987, 133 cases of ENANBH at a college in Sargodha, Pakistan were apparently caused by contamination of a water supply with feces from a nearby broken sewer line<sup>4</sup>. Sera and feces were studied by IEM to determine if this epidemic was caused by, and if patients excreted, HEV. Paired sera from a Sargodha patient were tested for antibody to known HEV particles from Nepal. Anti-HEV was detected in the convalescent-phase serum but not in the acute-phase serum, an unusual finding because antibody ratings by IEM are typically higher in the acute phase of ENANBH. Ninety five fecal specimens were examined for the presence of virus-like particles (VLPs). One of 48 (2%) feces collected during early nonspecific symptoms had VLPs; this specimen was among those from 21 patients who subsequently developed elevated serum ALT levels. Ten of 47 (21%) acute-phase feces had VLPs. Positive specimens were collected within 7 days after the onset of jaundice or dark urine, and the concentration of VLPs was usually low. The identity of the VLPs with HEV was confirmed by their reaction with serum from a chimpanzee infected with HEV from India<sup>1</sup>. We concluded that at least one patient had a serologic response to infection with HEV. In addition, HEV was detected in a small proportion of feces from early in the acute phase of ENANBH but, unexpectedly, was less frequently detected in feces from prior to the acute phase.

<sup>1</sup>Arankalle VA, Ticehurst J, Sreenivasan MA, Kapikian AZ, Popper H, Pavri KM, Purcell RH. *Lancet* i:550-4, 1988; <sup>2</sup>Bradley D, Andjaparidze A, Cook EH Jr, McCausland K, Balayan M, Stetler H, Velazquez O, Robertson B, Humphrey C, Kane M, Weisfuse I. *J Gen Virol* 69:731-8, 1988; <sup>3</sup>Purcell RH, Ticehurst JR. In Zuckerman A (ed): *Viral Hepatitis and Liver Disease*. Alan R Liss, 1988, p 131-7; <sup>4</sup>Iqbal M, Ahmed A, Qamar A, Dixon K, Duncan JF, Rauf A, Malik I, Legters L. Submitted for publication.

W: SCHISTOSOMIASIS - IMMUNOLOGY

433 IgE MONOCLONAL ANTIBODY TO SCHISTOSOMA MANSONI: SPECIFICITY, PARTIAL PURIFICATION OF ANTIGEN, AND APPLICATION FOR ANTIGEN DETECTION.

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U.S. Naval Medical Research Unit #3, Cairo, Egypt, and Specialty Laboratories, Inc.,  
Santa Monica, CA.

A murine IgE hybridoma was produced by fusion of P<sub>3</sub>x63 Ag8 plasmacytoma with spleen cells from NMRI mice immunized intraperitoneally with freeze-thaw antigen from adult S. mansoni. The positive clone was detected by passive cutaneous anaphylaxis assay (PCA) in Sprague-Dawley rats. The ascitic fluid of the positive clone was injected into the balb/c mice. The reactivity of this monoclonal antibody with other helminthic antigens such as adult Fasciola gigantica, Trichinella spiralis and Echinococcus granulosus was tested by PCA and found to be negative. To characterize the antigenic specificity of this monoclonal antibody, different preparations of S. mansoni antigens were tested; only soluble egg antigen (SEA) gave positive results. SEA was fractionated sequentially using concanavalin A-sepharose, Sephadex G-100 and DEAE cellulose chromatography. The reactive fraction is of high molecular weight (elutes in void volume of Sephadex G-100), contains no mannose or glucose, and elutes with 0.5M NaCl from a DEAE cellulose column. SDS-PAGE shows reactivity with several different molecular weight proteins ranging from 25-100 kilodaltons. This monoclonal antibody was used for ELISA detection of S. mansoni antigen in schistosomiasis patients' sera; more than 80% of chronic schistosomiasis patients gave positive results. (Partially supported by Work Unit #3M162770A870AQ126 NMRDC Bethesda, MD).

434 ROLE OF CLONED T LYMPHOCYTE SUBSETS IN SCHISTOSOMULE KILLING  
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Immunity in experimental schistosomiasis either antibody or cell-mediated is dependent on T lymphocytes. T cell clones were generated from C57Bl/6J mice vaccinated with irradiated Schistosoma mansoni cercariae. After limiting dilution and expansion, 8 clones were chosen for study. Eight are Thy 1.2 positive; 6 are L3T4 positive and 2 are Lyt-2 positive. All are responsive to schistosomule antigen by proliferation and all produce lymphokines or interleukins. Of the L3T4 positive clones, 3 are thought to be T<sub>H</sub>1 cells by their Interleukin-2 (Il-2) production. Three others produce Interleukin-4 (Il-4) and are thought to be T<sub>H</sub>2 cells. Lymphokine production was assessed using the HT-2 cell line and anti Il-2 or anti Il-4. In assays for killing schistosomules, Thy 1.2 and C' treated peritoneal cells from vaccinated mice were placed in 96 well plates with different clones (35,000 T cells/well) with and without heat-inactivated vaccine serum. After 72 hours, killing was assessed by .15% methylene blue uptake. Wells containing T<sub>H</sub>2 cells generated large numbers of mast cells which then attached to worms even when no immune serum was present. There was no significant increase in killing in any T<sub>H</sub>2 wells. Wells containing T<sub>H</sub>1 cells showed no increased killing without serum, but when immune serum was present there was 28-42% killing beyond controls and increased numbers of macrophages adhering to the worms. Further studies are being done with antilymphokines to investigate this phenomenon.

W: SCHISTOSOMIASIS - IMMUNOLOGY

- 435 THE REGULATION OF IMMUNITY IN SCHISTOSOMIASIS BY AN IDIO-TYPICALLY AND GENETICALLY RESTRICTED T-CELL DERIVED SUPPRESSOR-EFFECTOR FACTOR  
P.J. Perrin\*, L. JiaoJiao, and S.M. Phillips. University of Pennsylvania, School of Medicine, Philadelphia, PA.

Previous studies from our laboratory have established a role for anti-schistosome specific T cells in regulating the development of the protective immune response. For example, immunization with anti-schistosome-directed T blasts can partially ablate the development of protective immunity thru the stimulation of an anti-clonotypic T cell response. To investigate the mechanisms of this regulatory response, we incubated T cells derived from spleens of infected mice with Soluble Cercarial Immunogen (SCI) for 18 hr and collected the supernatants from these cultures after a further 48 hr. These supernatants contained an activity (TseF) which suppressed the DTH response against challenge with SCI in an antigenically specific manner. Furthermore, TseF, produced by H-2<sup>D</sup> cells, but, by H-2<sup>K</sup> cells suppressed H-2<sup>D</sup> reactivity. The factor had no effect upon *in vitro* antigen mediated blast transformation. Five weeks after exposure to irradiated cercariae mice were injected with either antigenically homologous or heterologous TseF, 2 days before, 1 day before, and 1 day after challenge with normal *S. mansoni* cercariae. The mice were perfused 7 weeks later to determine parasite development. Those animals receiving the homologous anti-schistosome TseF had a lower functional protective immunity than those which received the control factor (mean reduction 39%). It appears that soluble mediators, analogous to secreted T cell receptors are operative in schistosomiasis. A characterization of these mediators, both functionally and structurally, will allow for a better understanding of their regulatory mechanisms.

- 436 PARTIAL PURIFICATION AND IDENTIFICATION OF EOSINOPHIL STIMULATION PROMOTER: A COMBINATION OF GM-CSF AND IL-5.  
W.E. Secor\*, S.J. Stewart, and D.G. Colley. Vanderbilt Univ. Sch. of Medicine and VA Medical Center, Nashville TN. 37212

Eosinophil stimulation promoter (ESP), a murine lymphokine which induces eosinophil migration, is produced by spleen cells from mice infected for 8 wks with *Schistosoma mansoni* upon stimulation with concanavalin A or schistosome soluble egg antigens (SEA). Partial chromatographic purification of ESP reveals that fractions containing ESP activity also contain granulocyte/macrophage colony stimulating activity (GM-CSF). FPLC gel filtration and anion exchange analysis of murine (Mu) GM-CSF yields activity that co-elutes with partially purified ESP. Analyses of various cytokines indicate that recombinant (r) MuGM-CSF and rMuIL-5 each exert low level ESP activity, and act in an additive manner when both are present. Purified MuIL-3; rMuIL-2, rMuIL-4, rMuTNF-alpha; r-rat IFN- $\gamma$ ; and purified human IL-1 have no activity in ESP assays. Culture supernatants made in the presence of cyclosporin A (an inhibitor of production of IL-2, IL-3, and IFN- $\gamma$ ), but not GM-CSF have ESP activity but no IL-2 activity. Multiple, effective anti-GM-CSF absorption procedures lower, but do not eliminate ESP activity. It seems GM-CSF and IL-5 are each partially responsible for, and contribute to, ESP activity produced by concanavalin A- or SEA-stimulated spleen cells. Current studies focus on the differential involvement of T helper cell subsets in eosinophil responses.

(Supported in part by NIH grant AI-11289 and the VA)

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- 437 A SERUM FACTOR SUPPRESSES TNF-MEDIATED ACTIVATION OF HUMAN EOSINOPHIL TOXICITY TO SCHISTOSOMULA OF S. MANSONI.  
David S. Silberstein,\* Marjorie S. Minkoff, and John R. David.  
Harvard Medical School and Brigham and Women's Hospital, Boston, MA.

Recombinant tumor necrosis factor (TNF) enhanced the antibody-dependent toxicity of human eosinophils to schistosomula of S. mansoni in vitro (in 56 experiments, using eosinophils from 19 different subjects), demonstrating a potential in vivo role for TNF in resistance to schistosomiasis. During these studies, we identified a human subject whose eosinophils failed to respond to TNF with enhanced cytotoxicity in 8 of 10 experiments. In the two experiments when there was a response to TNF, the blood was drawn when the subject was undergoing severe contact dermatitis reactions. This suggests that eosinophil responsiveness to TNF is under immunological control. At concentrations as low as 2%, serum from the unresponsive subject prevented heterologous eosinophils from responding to TNF. Of 122 other sera that were tested, 5 had similar cross-inhibitory activity. When chromatographed on a TSK-400 HPLC sizing column, the inhibitor was recovered in fractions equivalent to a molecular weight of approximately 80,000. The inhibitor was destroyed by heating at 80°C or by treatment with trypsin. Sizing chromatography increased the activity of the inhibitor by a factor of 50 to 2000, suggesting that size fractionation removes a stabilizing component or counter-inhibitor. When activated inhibitor was added back to serum or to other sizing fractions, the activity did not decrease. Thus the activation of the inhibitor is irreversible. Lower levels of inhibitor were derived from each of seven different control sera by chromatography. Thus, the inhibitor is probably present in an inactive form in all sera, but in a partially activated form in the serum of the TNF-unresponsive subject and in 4% of the sera tested. This inhibitor may be the cause of unresponsiveness to TNF observed in eosinophils from the TNF-unresponsive subject and may be part of an immunological regulatory mechanism to suppress the pro-inflammatory activities of TNF.

- 438 FRACTIONATED SERA FROM SCHISTOSOMA MANSONI INFECTED PATIENTS CONFERS PASSIVE PROTECTION IN MICE. J. Jwo and P.T. LoVerde\*. Department of Microbiology, State University of New York, Buffalo, NY.

Previous studies have shown that protection against challenge infection with S. mansoni cercariae can be passively transferred to mice with monoclonal antibodies and sera from animals immunized with attenuated cercariae. However, the passive transfer of immunity from sera of animals including humans with chronic S. mansoni infections has not been successful. We report the passive transfer of immunity by human chronic infection sera (CHS) affinity purified against the surface membrane antigens of schistosomula. Sera obtained from infected Egyptian patients was fractionated by affinity chromatography using membrane antigens of 3 hr schistosomula coupled to sepharose. Both unbound (UB) and bound (B) fractions which contained IgG and IgM isotypes were characterized by ELISA, immunofluorescence and in vitro killing assays. The B fraction recognized NP-40 extracts of 3 hr schistosomules in ELISA and showed surface fluorescence on the surface of 3 hr live schistosomules, whereas the UB fraction did not. The B fraction mediated 95% killing of schistosomula in a complement dependent in vitro assay, the UB fraction and unfractionated CHS did not mediate killing. In passive immunization, the B fraction provided about 30% passive protection in mice when injected at 1 day or 6 days after challenge, 20% protection at 15 days but failed to mediate passive protection when administered 24 days after challenge. However, CHS failed to mediate passive protection. Our data suggest that some factors in the UB fraction other than blocking antibodies may account for the inability of CHS to confer protection (funded by AI22567).

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- 439 Lymphoid phenotypic alterations in murine schistosomiasis mansoni; with and without suppressor factor administration. G.S. Henderson,\* T.L. McCurley, and D.G. Colley. Vanderbilt Univ. Sch. Med. and V.A. Med. Ctr., Nashville, TN.

The phenotypic distribution of thymocytes, spleen (SPL) and lymph node (LN) lymphocytes in mice during infections with Schistosoma mansoni was analyzed by flow cytometry. Cells from mice infected for 8, 12 and 20 weeks were studied with a panel of monoclonal and polyclonal antibodies and lectins to evaluate the dynamics of phenotypic alterations at times of maximal anti-egg granuloma formation and modulation. Relative to the cell numbers in normal, uninfected mice, SPL and LN of infected mice had increases of 900% and 500% more PNA+ lymphocytes, respectively. Both SPL and LN had increases of 400-500% B lymphocytes, and 80% Thy 1.2+ lymphocytes. At 8 and 20 wks, respectively, L3T4/Lyt-2 ratios decreased in SPL from 4.3 to 2.4, due to an absolute increase in Lyt-2+ cells. The parallel L3T4/Lyt-2 ratio in LN dropped from 3.4 to 1.6, due to an absolute increase in Lyt-2+ cells and decrease in L3T4+ cells. No relative thymocyte subpopulation changes were related to the presence or duration of infection. Treatment of 6-8 wk-infected mice with a soluble T suppressor factor (SmTsF) lead to modulated granulomas, but did not induce phenotypic profiles characteristic of mice with chronic infections. The data suggest that systemic lymphocyte phenotypic changes observed in chronic infection are neither directly induced, nor necessary, for SmTsF-induced suppression.

(Supported in part by NIH grant AI-11289 and the VA)

- 440 Mechanism of schistosomulum killing by lymphokine-activated macrophages. S.L. James and J. Glaven. George Washington University Medical Center, Washington, D.C. 20037

Schistosomula of S. mansoni are killed by murine peritoneal macrophages activated by prior exposure to recombinant-derived gamma interferon ( $r\text{INF}\gamma$ ) or  $\text{INF}\gamma$ -containing lymphokine (LK) preparations (James, Immun. Res. 5:139, 1986). Macrophage larvacidal activity is contact-dependent and requires protein synthesis and secretion, but appears to be independent of the activities of oxidative metabolites, thymidine, arginase and lysosomal enzymes (James & Glaven, Inf. Immun. 55:3174, 1987). Recent studies suggest that the monokine tumor necrosis factor (TNF) is directly involved in larval killing by LK-activated macrophages. Antibodies to TNF inhibit macrophage-mediated schistosomulum killing by 40-60%. Moreover,  $\text{ZnSO}_4$  (which inhibits the tumor cytotoxicity of TNF) inhibits macrophage larvacidal activity by up to 85%. Recombinant-derived human or murine TNF is toxic to schistosomula at high concentrations, and, upon gel filtration, the larvacidal activity in tumor necrosis sera prepared by endotoxin challenge of BCG-infected mice co-elutes with TNF tumoricidal activity. Finally, TNF is present in larvacidal supernatant fluids collected from the macrophage cell line IC-21 following LK, endotoxin and A23187 stimulation. Macrophage larvacidal activity is also inhibited by  $\text{FeSO}_4$ , arginase or  $\text{N}^G$ -monomethyl-L-arginine. These studies suggest that TNF functions directly as a cytotoxic effector molecule of schistosomulum killing by LK-activated macrophages, and that the effect may involve metabolic inhibition by an arginine-dependent mechanism similar to that described by Hibbs et al with tumor cell targets.

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**TARGET ANTIGENS OF HOST ANTIBODIES INVOLVED IN THE CHEMOTHERAPY OF SCHISTOSOMA MANSONI WITH PRAZIQUANTEL (PZQ).** P. Brindley M. Strand & A. Sher, LPD, NIAID, Bethesda, MD and Johns Hopkins University, Baltimore, MD.

To identify target antigens of antibodies involved in synergy between PZQ and the immune response in chemotherapy of schistosomiasis, 6 wk old worms perfused from immunodeficient, athymic mice 1h after PZQ-treatment were assayed by indirect immunofluorescence assay (IFA) for reactivity with antibodies recognizing tegumental components. Only 5 of the 26 antibodies tested reacted, indicating that damage caused by PZQ to the tegument is localized anatomically. Reactions with anti-schistosome carboxypeptidase and -alkaline phosphatase gave IFA patterns different from that observed when worms from 6 wk infected drug-treated, immunologically intact mice are stained with anti-mouse Ig. In contrast, two of three monoclonal antibodies recognizing different epitopes on a 200 kDa glycoprotein abundant in worm tubercles gave patterns similar to that seen in intact mice following PZQ administration. The biological importance of this anti-200 kDa reaction was confirmed by demonstrating that transfer of one of the positive monoclonal antibodies to 6 wk infected u-suppressed mice reconstitutes the efficacy of PZQ-treatment to its normal level. These results suggest that antibodies involved in synergy with PZQ react with a limited set of antigens and implicate the 200 kDa tubercle protein as a major target of this response in naturally infected hosts.

442 **FOUR MAJOR SURFACE MOLECULES OF SCHISTOSOMA MANSONI ARE ANCHORED TO THE SCHISTOSOMULUM MEMBRANE BY GLYCOSYLPHOSPHATIDYLINOSITOL (GPI).** E. J. Pearce and A. Sher. ICBS, LPD, NIAID, Bethesda, Md.

Schistosomula of *S. mansoni* were examined for the presence of GPI anchored surface membrane proteins. Parasites were surface iodinated and cultured in the presence or absence of phosphatidylinositol-specific phospholipase C (PIPLC). Culture supernatants were then analysed: 1, by ultracentrifugation to ascertain which molecules released from the surface are soluble or contained in membrane vesicles; 2, by immunoprecipitation with antibodies specific for the "cross reacting determinant" (CRD), an epitope revealed on GPI anchored proteins only after cleavage by PIPLC, and 3, by immunoprecipitation with sera from vaccinated or infected mice. Schistosomula were shown to possess 4 GPI anchored surface molecules of Mr's 70k, 32k-38k and 18k, which are spontaneously released from the surface of schistosomula in association with membrane, probably as a result of blebbing, but remain insoluble until cleaved by PIPLC. No endogenous PIPLC activity is evident in the parasites. The 32k-38k proteins are recognized by antibodies from mice vaccinated with irradiated cercariae as well as from infected mice. In addition, the 18k molecule is recognized by vaccinated but not infected mice while the 70k protein appears to be non-antigenic. Since the 18k, 32k and 38k molecules have previously been shown to be important parasite immunogens, these results suggest that GPI anchorage and membrane release may play an important role in the elicitation of host immune responses against the schistosome tegument.

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- 443 MODULATION OF EGG GRANULOMAS IN MICE CHRONICALLY INFECTED WITH SCHISTOSOMA MANSONI IS ASSOCIATED WITH DECREASED PRODUCTION OF FIBROBLAST STIMULATING FACTORS. S. Prakash\*, D.J. Wyler, and A.E. Postlethwaite+. New England Medical Center Hospitals, Inc., and Tufts University School of Medicine, Boston, MA, and University of Tennessee, Memphis, TN+.

Hepatic egg granulomas from mice infected with S. mansoni for 8 wks, when cultured in vitro elaborate factors that stimulate fibroblast growth, and synthesis of collagen, fibronectin and proteoglycans. We have proposed that these factors play a role in the pathogenesis of hepatic fibrosis, suggesting that a molecular link exists between egg granulomas and fibrosis in schistosomiasis. The liver of chronically infected mice (>16 wks) synthesize collagen at a reduced rate compared to that of more recently infected mice (8 wks). We therefore compared the production of fibrogenic factors by granulomas of mice infected for 8 wks with those from more chronically infected mice. We found that granulomas isolated at various times after 8 wks, progressively elaborate less of fibroblast growth stimulating activity in vitro. By 30 wks, no growth factor is detectable in culture supernatants of isolated granulomas. A reduction in the ability of granulomas to produce factors that stimulate fibroblast proteoglycan synthesis was also observed. These findings suggest that modulation of granulomatous inflammation in murine S. mansoni is also reflected by reduced production of granuloma-derived fibrogenic factors. Failure of down-regulation of this process in selected individuals might be an important determinant of subsequent severe hepatic fibrosis.

- 444 LYMPHOKINE MESSENGER RNA LEVELS IN SPLEEN TISSUE FROM PATIENTS WITH HEPATOSPLENIC SCHISTOSOMIASIS. M.D. Ricciardone\*, K.A. Kamal, M.M. Mansour, and J.N. Woody. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

The major pathology in schistosomiasis is liver fibrosis, which is caused by a T cell-mediated inflammatory response to schistosome eggs. In order to evaluate the role of lymphokines in the regulation of this inflammatory response, cloned cDNA probes were used to measure lymphokine messenger RNA (mRNA) expression in spleen tissue from patients with severe hepatosplenic schistosomiasis. Spleen tissue was homogenized in guanidium isothiocyanate and the RNA isolated by centrifugation through cesium chloride. RNA was denatured with formaldehyde, transferred to nitrocellulose using a Minifold II slot blot apparatus, and hybridized with radiolabelled cDNA probes. Hybridization was detected by autoradiography and quantitated by densitometry. Messenger RNA levels were normalized by hybridization with 7B6, a probe which recognizes an mRNA whose expression is invariant throughout the cell cycle. Lymphokine mRNA levels in spleen tissue from patients with schistosomiasis were then compared to lymphokine mRNA levels in spleen tissue from a trauma victim with no history of exposure to schistosomiasis. Patients with hepatosplenic schistosomiasis showed elevated levels of several lymphokines, including interleukin-2 (3.8-fold), interferon-gamma (3.2-fold), interleukin-1 alpha (2.2-fold), interleukin-4 (1.9-fold), and interleukin-3 (1.8-fold). Levels of interleukin-1 beta and tumor necrosis factor mRNA were similar in both patient and control spleens. Analysis of the lymphokine mRNA levels in liver tissue of these patients is in progress. (Supported by NMRDC, Bethesda, MD, Work Unit #61152N-MR00001-001-3083).

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- ACAV - American Committee on Arthropod Borne Viruses Annual Meeting, Wednesday, December 7, 1:30 PM, Diplomat.
- ACME - American Committee on Medical Entomology Annual Meeting, Thursday, December 8, 1:15 PM, Diplomat
- ACME BUS - ACME Business Meeting, Monday, December 5, 5:00 PM, Executive.
- ADVOCACY - Effective Advocacy Workshop, Monday, December 5, 5:30 PM, Palladian.
- ASTMH - American Society of Tropical Medicine and Hygiene Annual Business Meeting, Tuesday, December 6, 4:30 PM, Regency.
- BEAVER - Paul C. Beaver Symposium, Wednesday, December 7, 1:30 PM, Palladian.
- CMI - Cell-Mediated Immunity to Asexual Malaria Parasites Workshop, Thursday, December 8, 1:30 PM, Ambassador.
- CLIN GRP- Clinical Group Meeting, Monday, December 5, 1:30 PM, Diplomat.
- COMMEM - Tropical Medicine Commemorative Fund Lecture, Thursday, December 8, 11:00 AM, Ambassador.
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- EHRlich - Ehrlichiosis Symposium, Wednesday, December 7, 1:30 PM, Executive.
- LATE - Late Breaking Advances in Molecular Biology, Tuesday, December 6, 1:15 PM, Palladian.
- MAL IMMUN - Malaria Immunity in Mice and Humans, Monday, December 5, 1:15 PM, Palladian.
- PLENARY - Plenary Session, Monday, December 5, 8:40 AM, Regency.
- PRES ADDRESS - ASTMH Presidential Address by Jacob K. Frenkel, Tuesday, December 6, 3:30 PM, Regency.
- WATER - The Water and Sanitation Decade (1981-1990): Its Impact on Tropical Diseases, Tuesday, December 6, 10:00 AM, Regency.

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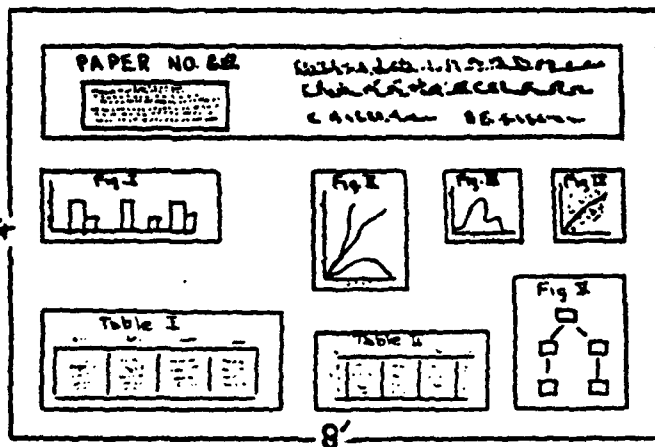
GUIDELINES FOR POSTER PRESENTATIONS

Abstracts scheduled for presentation in the poster session will be grouped by topic, numbered, and listed in the program.

The poster board surface area is 4' high and 8' wide. Prepare a label for the top of your poster space indicating the program number of your paper, its title and authors and their affiliations. The lettering for this section should be at least 2-3" high. A copy of your abstract should be posted in the upper left-hand corner of the poster board.

All illustrations should be made up beforehand. Bear in mind that your illustrations must be read by interested scientists from distances of about 3' or more. Charts, drawings and illustrations might well be similar to those you would otherwise use in making slides; profitably cruder and more heavily drawn. Illustrations reproduced photographically should be reproduced with a mat finish to avoid the glare produced by glossy prints. Simple use of color can add emphasis effectively. Do not mount illustrations on heavy board because it may be difficult to keep in position on the poster boards. Hand lettered material should contain appropriately heavy lettering at least 3/8" high. Shade block letters where possible. Typed material should be typed on a Bulletin (large type) typewriter, and photographically enlarged. Keep illustrative material simple. You might also find it useful to have on hand a tablet of suitable sketch paper (9"x12") as well as one or two felt marking pens.

"Poster Assistants" will help you with any information or technical assistance you may need. A suggested arrangement of poster is illustrated below.



Provide a label containing the abstract number, title, and name(s) of author(s) to identify your presentation easily.

Post copy of your abstract.

Prepare and bring with you to the meeting all illustrations needed for your presentation - figures, tables, schemes, equations, etc..

Mount your labels and illustrations on the fiberboard by means of drawing pins (thumb-tacks). These items will be provided in the area.

Please do not write or draw on the Poster Boards.

GUIDELINES FOR ORAL PRESENTATIONS

Time - Oral presentations in the regular scientific sessions may be up to 10 minutes in length, with an additional 5 minutes for discussion. The time allocated for oral presentations in symposia and workshops varies - as noted in the program.

Presentation - Please rehearse your presentation so it does not exceed the allotted time, and so that it is clearly coordinated with your slides. Giving the talk to several colleagues 1-2 weeks before the meeting is usually very helpful. It also allows enough time to revise slides that are unacceptable, and to make additional slides if they are needed.

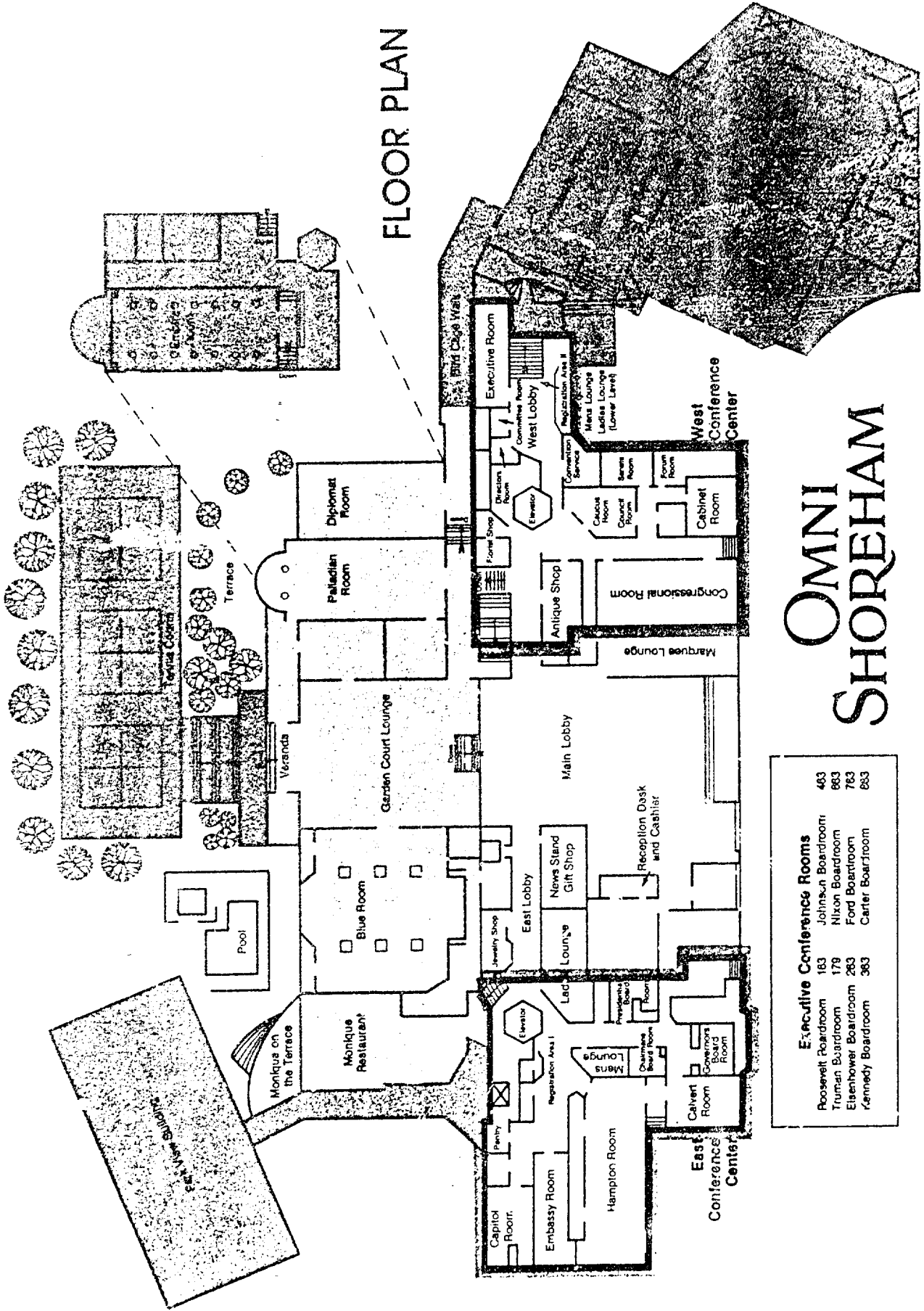
Organization - In addition to the data being presented, each talk should explain why the studies were performed (what question was being asked), and provide a clear summary of the results.

Slide Composition - Slides must be legible from the back of the room. In general, 8-9 lines (including title, headings and data) are the maximum for one slide. Any slide introduced by "I know this is crowded, but..." should be replaced.

Chairpersons - In fairness to the audience, chairpersons will ask speakers to explain the content of excessively crowded slides - rather than reviewing them line by line with the pointer.

To permit members to attend different sessions in the same morning or afternoon, chairpersons have been asked to limit discussion to 5 minutes, and to recess for 15 minutes if a speaker does not show for his/her presentation.

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# OMNI SHOREHAM

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