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PRINCIPAL INVESTIGATOR: Robert E. Shope, M.D.

CONTRACTING ORGANIZATION: Yale University School of Medicine
333 Cedar Street
P.O. Box 20846
New Haven, Connecticut 06510-8047

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Robert E. Shope, M.D.

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Yale University School of Medicine
333 Cedar Street
P.O. Box 20846
New Haven, Connecticut 06510-8047

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Rabbits were immunized successfully intravenously with Ross River, Germiston, and Japanese encephalitis viruses using immunogens grown in RK-13 rabbit kidney cells. These animals were boosted 2 months after the initial immunization. Titers in ELISA for optimal coating of the solid phase ranged between 1:500 and 1:64000.

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SUMMARY

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FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

In conducting research using animals, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Robert E. Shope May 20, 1994

PI Signature Date

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BODY OF REPORT

1. Production of mouse brain sucrose-acetone extracted antigens.

Five antigens were prepared during the year as listed here:

Antigen	Strain	Passage	Number of lots	Volume to date (ml)
Germiston	SA AR1050	sm17/19/20	6	473
Qalyub	EgAr 370	sm4	6	229
Sicilian	Sabin	sm37,Vero2	1	23
VS-Indiana	Ind. Lab	sm7	1	45
Ganjam	IG 619	sm5	1	67

Additionally, 22 viruses were passaged in baby mice awaiting preparation of antigen lots. These were lymphocytic choriomeningitis (4 lots), Maguari (5 lots), Mayaro (2), O'nyong-nyong (2), Qalyub (4), Rocio (3), Tataguine (1), tick-borne encephalitis (2), vesicular stomatitis-New Jersey (4), vesicular stomatitis-Indiana (6), West Nile (1), Zika (8), Belterra (4), Bunyamwera (3), Ganjam (5), Hazara (9), Hughes (2), Ilheus (2), Ilesha (7), Jamestown Canyon (1), Japanese encephalitis (1), and LaCrosse (1).

2. Production of antibody to arboviruses in rabbits.

Rabbits were immunized to Japanese encephalitis, Hazara, Bhanja, Ilheus, Qalyub, Bwamba, Oropouche, Germiston, and Ross River. It was found that rabbits responded with much higher titers when they were rested for two months after the initial series of 3 iv inoculations, then boosted iv and bled one week later.

Results of initial testing were:

Virus	RK-13 titer	Optimal titer in ELISA	Volume (ml)
Ross River	3 log LD50	1:500	25
Germiston	>4.5 log LD50	1:1000	37
		1:16000	38
Japanese enc.	>4.5 log LD50	1:1000	33
		1:64000	13

Attempts to immunize rabbits with C6/36 cell grown virus were associated with non-specific background in ELISA.

DISCUSSION AND CONCLUSIONS

Two problems merit discussion:

1. Initial success in adapting arboviruses to grow in RK-13 cells has not held up. Approximately half of the viruses do not appear to grow. Further, the tick-borne agents as a rule do not grow in the proposed alternative system, C6/36 cells, and non-specific background in ELISA has been observed.

2. A much more pervasive problem involves the senior technician employed to do the mouse and rabbit inoculation, and the processing of antigens and ammonium sulfate concentration of IgG. He became ill during the first month of this project year. He was diagnosed as having carcinoma of the colon and underwent surgery. The tumor had metastasized and he was left with a colostomy. He was entitled to remain on full pay for a 6-month period because of accumulated sick leave and vacation and the project was without technical help for the remainder of the contract period. An Associate in Research is actively being sought to fill the position now, but the work is significantly behind schedule.