AD-A061 064 ALABAMA UNIV IN BIRMINGHAM F/G 6/13
GENETIC AND MOLECULAR STUDIES OF THE PHLEBOTOMUS FEVER GROUP OF--ETC(U)
AUG 78 D H BISHOP DAMD17-78-C-8017
UNCLASSIFIED NL

1 OF 1
AD A061 064

END
DATE FILMED
2-79
DDC

REPORT NUMBER 1

(12) LEVEL II

GENETIC AND MOLECULAR STUDIES OF THE PHLEBOTOMUS FEVER GROUP OF VIRUSES

ANNUAL REPORT

DR. DAVID H. L. BISHOP

AUGUST 1978

Supported by

U. S. ARMY MEDICAL RESEARCH AND
DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD-17-78-C-8017

University of Alabama
Birmingham, Alabama 35294

DDC DISTRIBUTION STATEMENT

Approved for public release;
distribution unlimited

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
Rept. no. 1 (Annual)	17 Feb - 31 Aug 78	

4. TITLE (and Subtitle)
GENETIC AND MOLECULAR STUDIES OF THE PHLEBOTOMUS FEVER GROUP OF VIRUSES.

5. TYPE OF REPORT & PERIOD COVERED
Annual Report
2/1/78 - 8/31/78

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
DR. DAVID H. L. BISHOP

8. CONTRACT OR GRANT NUMBER(s)
DAMD-17-78-C-8017

9. PERFORMING ORGANIZATION NAME AND ADDRESS
University of Alabama
Birmingham, Alabama 35294

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
61102A
3M161102BS01 00.028.

11. CONTROLLING OFFICE NAME AND ADDRESS
US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

12. REPORT DATE
August 1978

13. NUMBER OF PAGES
16

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)
16p.

15. SECURITY CLASS. (of this report)
UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Virus vaccines
Phlebotomus fever viruses
Genetic Recombination
Virus antigen

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The reporting period represents 6 months since the inception of this project. During this time we have directed our efforts in two main directions which relate directly to the objectives of the contract, which is to determine the recombination potential of the Phlebotomus fever group viruses. These two directions are (1) the selection of viruses and temperature limits for genetic recombination analyses, and (2) characterization of the major elements of selected viruses at the molecular level. (continued on back)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

387757

1. With regard to the first direction, for all the viruses we have on hand, we are investigating the temperature ranges for plaque assays and optimal plating conditions. These studies are still in progress.

2. With regard to the second direction, with Karimabad, Punta Toro, Chagres and the Sicilian sandfly fever virus, we have characterized to some extent their genomes and virion proteins. All four viruses have tripartite RNA genomes. For Karamabad and Punta Toro viruses, we have shown that their three viral RNA species (large, L, medium, M, and small, S) have unique oligonucleotide fingerprints. All four viruses have two or three major viral polypeptides, one or two of which are glycoproteins (G1 and G2), while the third is not glycosyated. Electron microscopic evidence indicates that the virus particles are spherical and enveloped with an outer layer of surface spikes.

I. SUMMARY

The reporting period represents 6 months since the inception of this project. During this time we have directed our efforts in two main directions which relate directly to the objectives of the contract, which is to determine the recombination potential of the Phlebotomus fever group viruses. These two directions are (1) the selection of viruses and temperature limits for genetic recombination analyses, and (2) characterization of the major elements of selected viruses at the molecular level.

1. With regard to the first direction, for all the viruses we have on hand, we are investigating the temperature ranges for plaque assays and optimal plating conditions. These studies are still in progress.

2. With regard to the second direction, with Karimabad, Punta Toro, Chagres and the Sicilian sandfly fever virus, we have characterized to some extent their genomes and virion proteins. All four viruses have tripartite RNA genomes. For Karimabad and Punta Toro viruses, we have shown that their three viral RNA species (large, L, medium, M, and small, S,) have unique oligonucleotide fingerprints. All four viruses have two or three major viral polypeptides, one or two of which are glycoproteins (G1 and G2), while the third is not glycosyated. Electron microscopic evidence indicates that the virus particles are spherical and enveloped with an outer layer of surface spikes.

II. REPORT

A. Introduction.

The objectives of this report are to determine the genetic capacity of Phlebotomus fever group viruses and the implications of forming new Phlebotomus fever virus genotypes. Since members of this group of viruses cause illnesses in epidemic proportions and therefore are of military significance, our objectives relate to the question of deriving vaccine strains of viruses which will be useful in protecting military and civilian personnel against virus infections.

To realize these objectives we aim to develop genetic tools in the form of temperature sensitive mutants of particular member viruses, and use these in mixed virus infections to produce new virus genotypes presumably by RNA segment reassortment (Gentsch & Bishop, 1976; Gentsch, et al., 1977b). By knowing the RNA segment coding assignments, we will be able to produce custom genotypes containing particular genetic information and gene products. Such genotypes will eventually be tested for their vaccine capabilities in model animal systems, and if effective, similar procedures to derive reassortant viruses will be used in clean cell systems in order to obtain virus vaccines that can be used to immunize man.

This report therefore includes both analyses of the genetic potential of selected Phelbotomus fever group viruses, and initial molecular studies to characterize the viral genomic RNA species and their gene products.

1. Genetic studies.

We have received from WRAIR, cloned virus samples of the following viruses belonging to the Phlebotomus fever serogroup: Candiru, Chagres, Itaporanga, Karimabad, Naples sandfly fever virus, Punta Toro, and two isolates of the Sicilian sandfly fever virus. In addition we have received from Dr. R. Shope, Icoaraci virus, as well as other stocks of the above viruses.

Each virus has been passaged in Vero cells to produce working stocks and titered in Vero cells, giving stock virus titers (Table 1) ranging from 4.3×10^6 (Naples sandfly fever virus) to 5.3×10^8 (Punta Toro).

A variety of plaquing conditions are being investigated for the viruses listed in Table 1. The procedures used to plaque Phlebotomus fever group viruses on Vero cells, although

useful at 35°C, result in cell death when the incubation temperature is raised to 39.8°C. The agent responsible for cell death appears to be the DEAE dextran which is included in the overlay medium. In our hands its inclusion is only marginally useful at 35°C. Without DEAE dextran the cell monolayer remains alive at least 7 days at 39.8°C.

Plaque assays at various temperatures are being run to define the plaquing capabilities of the viruses and thereby select a nonpermissive temperature for genetic studies. Such assays are being run on (1) original virus stocks and (2) high temperature passaged virus stocks, in case the original virus stocks represent temperature-sensitive (ts) mutants of an earlier wild type virus. These studies are in progress and not yet complete. Two intermediate results are that all the viruses produce plaques at 35°C and 38°C, but do not give plaques at 39.8°C. For Punta Toro virus, a 39.8°C passaged virus stock gives plaques at 39.8°C with a 39.8°C/35°C E.O.P. of 0.3. We are currently investigating intermediate temperatures (between 38°C and 39.8°C) for a suitable non-permissive temperatures for ts mutants (but permissive for the original wild-type virus stocks).

TABLE 1

Virus	Virus stock titers, pfu/ml (assayed in Vero cells at 35 C)	Day of stain overlay
Candiru (prototype)	2.0×10^7	6
Chagres (prototype)	2.4×10^8	5
Itaporanga (prototype)	7.0×10^7	6
Karimabad (prototype)	6.5×10^7	6
Naples sandfly fever virus (prototype)	4.3×10^6	7
Punta Toro (prototype)	5.3×10^8	5
Sicilian sandfly fever virus (prototype)	1.0×10^7	7
Sicilian sandfly fever virus (Tesh)	2.2×10^7	7

Each virus was titered to limit dilution in Vero cells, grown at 35° and overlaid with stain overlay on day indicated. Plaques (1 - 3mm) were read 12 h to 1 d. later. The overlay used for plaquing at 35°C consists of 0.9% agar, Medium 199, 10% (v/v) heat inactivated MEM fetal calf serum, 1% DMSO, fortified with essential amino acids and vitamins. The presence of DEAE dextran (20µg/ml) is optional.

2. Biochemical studies.

We have made considerable progress in defining the genome and virion polypeptides of several Phlebotomus fever group viruses. The results are summarized below.

a. Karimabad (KAR) virus.

(i) Viral RNA.

When the viral RNA is extracted from ^{32}P labeled Karimabad virus preparations and resolved by SDS-sucrose gradient centrifugation, three peaks of radioactivity are resolved. The resolution obtained is shown in Fig. 1. For convenience the three RNA species are designated large (L), medium (M) and small (S).

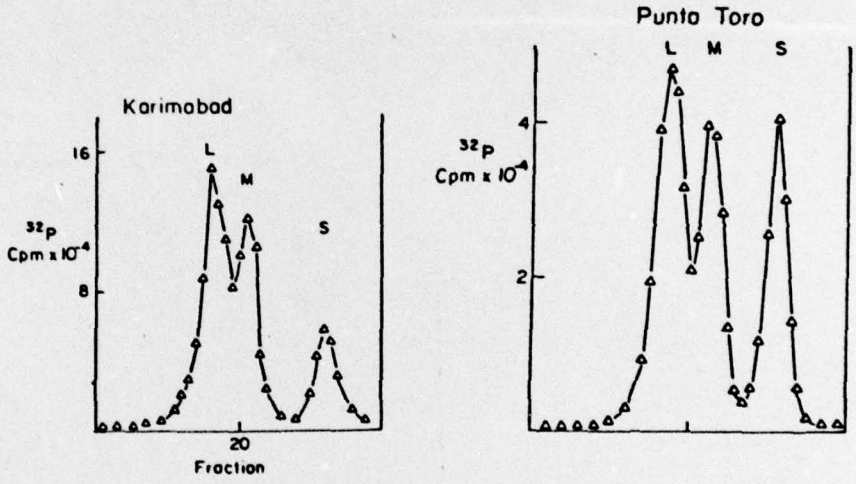

Fig. 1. SDS sucrose gradient centrifugation of KAR and PT viral RNA.

After recovery of the individual viral RNA species and digestion with ribonuclease T_1, the resulting oligonucleotides have been resolved by two dimensional polyacrylamide gel electrophoresis as described by Clewley, et al., 1977. The fingerprints obtained are shown in Fig. 2. They indicate that the three KAR viral RNA species each have unique and distinguishable nucleotide sequences, suggesting that they code for different gene products.

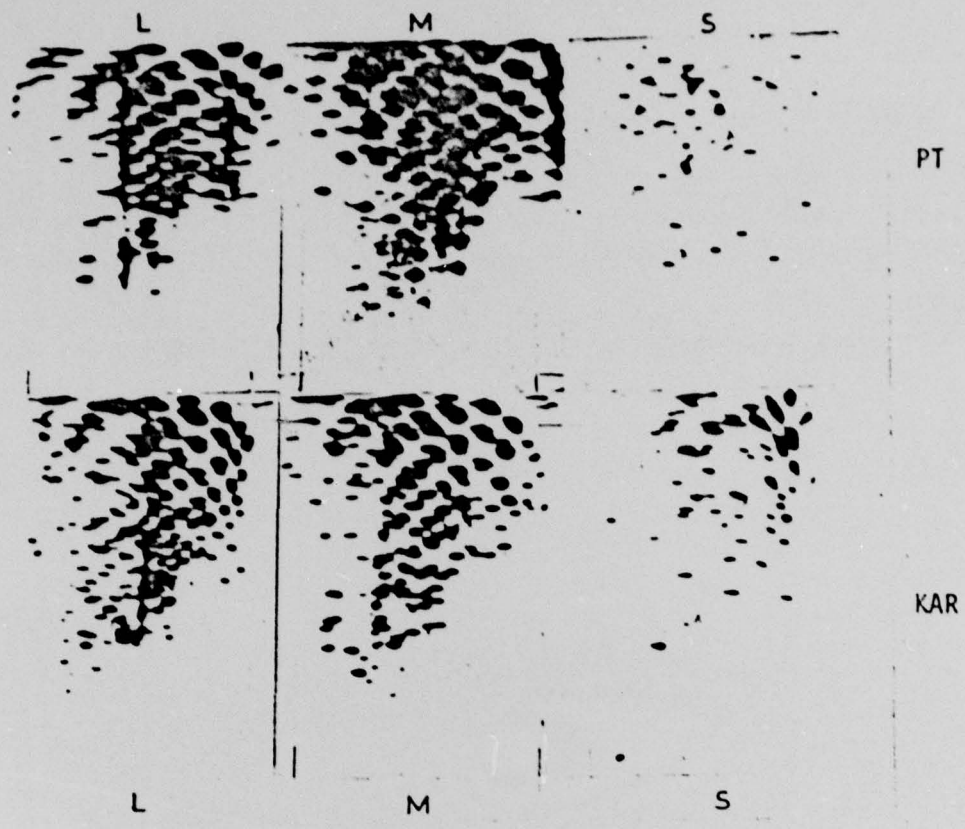

Fig. 2. Oligonucleotide fingerprint analyses of KAR and PT L, M, and S viral RNA species.

In order to obtain size estimates of the L, M, and S viral RNA species of KAR virus, aliquots of a ^{3}H uridine labeled KAR viral RNA preparation have been coelectrophoresed with aliquots of ^{32}P labeled BHK ribosomal RNA. The results are shown in Fig. 3.

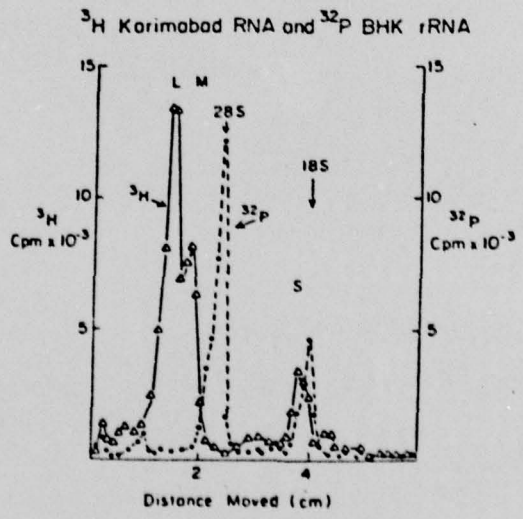

Fig. 3. Coelectrophoresis of ^{3}H KAR viral RNA with ^{32}P BHK ribosomal 28S and 18S RNA. The L, M and S RNA species of KAR virus are indicated.

A similar coelectrophoresis of ^{3}H uridine labeled KAR viral RNA with ^{32}P labeled viral RNA of snowshoe hare (SSH) virus (Fig.4), indicates that the L, M and S RNA species of KAR virus have apparent molecular weights of 2.5 x10^{6}, 2.1 x10^{6} and 0.65 x10^{6} respectively.

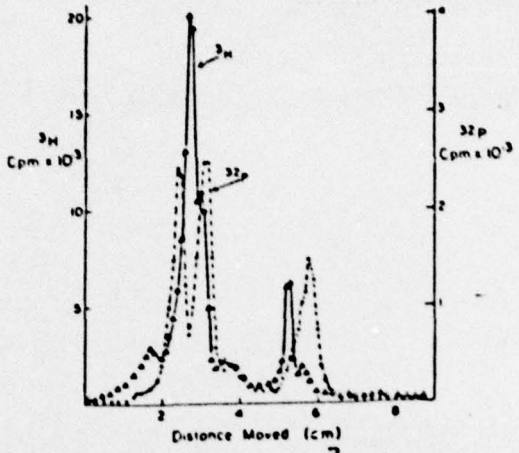

Fig. 4. Coelectrophoresis of ^{3}H KAR viral RNA and ^{32}P labeled SSH viral RNA species.

In summary, these analyses indicate that KAR virus has a tripartite genome with unique nucleotide sequences for all three RNA segments. The apparent molecular weights of the three viral RNA species are significantly different from those of other bunyaviruses (Gentsch, et al, 1977a).

KAR viral polypeptides.

When a ^{3}H leucine labeled virus preparation of KAR virus is coelectrophoresed under non-reducing conditions with a ^{14}C amino acid preparation of SSH virus, at least three major viral polypeptides are resolved (Fig. 5). They have apparent molecular weights of 100 x10^{3}, 75 x10^{3} and 24 x10^{3}.

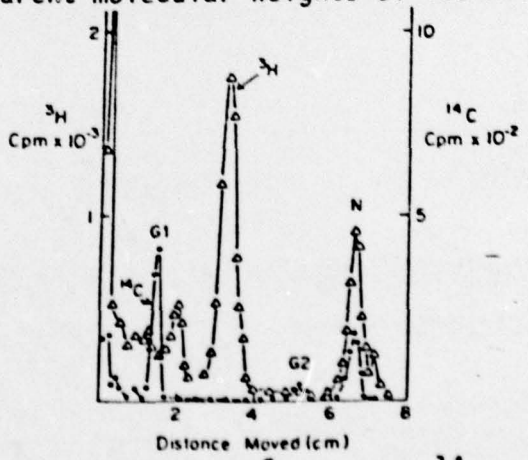

Fig. 5. Coelectrophoresis of ^{3}H KAR and ^{14}C SSH viral polypeptides.

Polyacrylamide gel electrophoresis of non reduced ^{3}H-glucosamine and ^{14}C-amino acid labeled KAR virus preparations indicates that the largest two viral polypeptides are glycosylated (Fig. 6). The largest glycoprotein has a higher ^{3}H to ^{14}C ratio than the smaller glycoprotein.

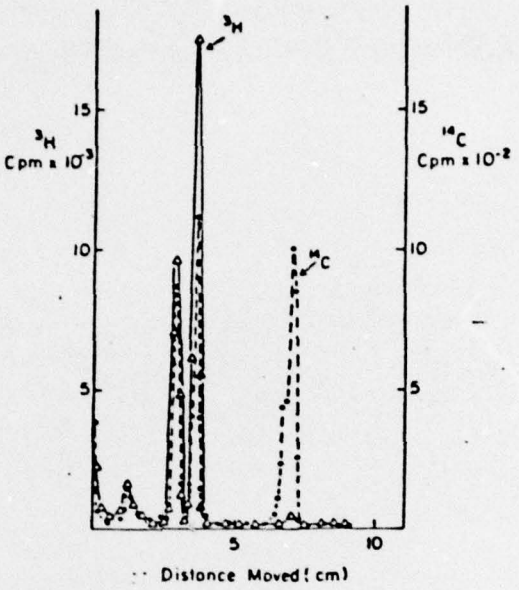

Fig. 6. ^{3}H-glucosamine and ^{14}C-amino acid labeled non-reduced KAR polypeptides.

After treatment with 1% mercaptoethanol, a single glycosamine polypeptide band is obtained (Fig. 7). Whether a minor band in the region of the small molecular weight unglycosylated polypeptide is enhanced after reduction of the viral polypeptides is not certain. This is currently being investigated.

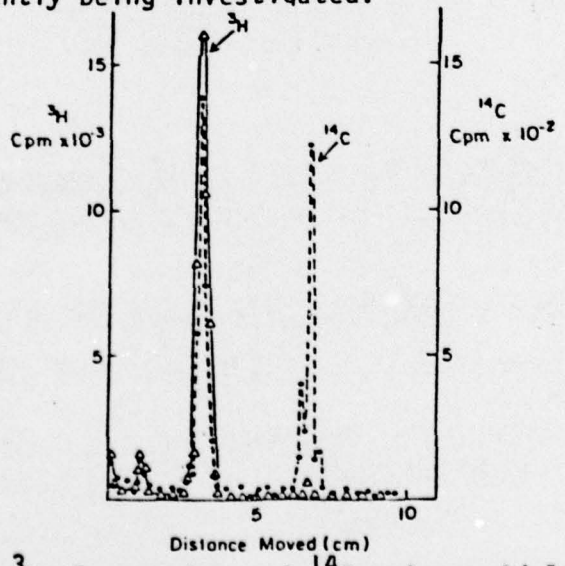

Fig. 7. ^{3}H-glucosamine and ^{14}C-amino acid labeled reduced KAR polypeptides.

(iii) Electron microscopy of KAR virus particles.

Electron micrographs of negatively stained purified KAR virus particles are shown in Fig. 8. For these preparations the virus was purified through two successive glycerol-tartrate gradients and dialysed against 0.01 M tris buffer, pH 7.2. Many of the virus particles (~ 90nm diameter) were found clumped together.

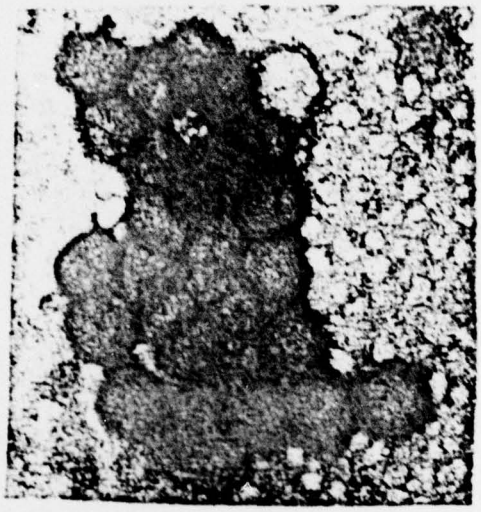

Fig. 8. Electron micrographs of negatively stained KAR virus preparation.

b. Punta Toro (PT) virus.

(i) Viral RNA species.

Resolution of the viral RNA extracted from ^{32}P labeled PT virus preparations by sucrose gradient centrifugation has given 3 peaks of radioactivity corresponding to a large (L), medium (M) and small (S) RNA species (Fig. 1). The L,M, and S RNA oligonucleotide fingerprints of PT virus have been obtained. They are clearly unique and differ from those of KAR or other bunyaviruses we have analysed. Again the analyses of PT RNA species indicates that each type of RNA has a unique nucleotide sequence.

Coelectrophoresis of ^{3}H uridine labeled PT viral RNA species with ^{32}P labeled BHK ribosomal RNA (Fig. 9), or ^{32}P labeled SSH viral RNA (Fig. 10), indicates that the L, M and S RNA species of PT virus have apparent molecular weights of 2.5 x10^{6} (L), 1.5 x10^{6} (M) and 0.6 x10^{6} (S). These values are not only signifigantly different from those of SSH, but also different from those of KAR virus.

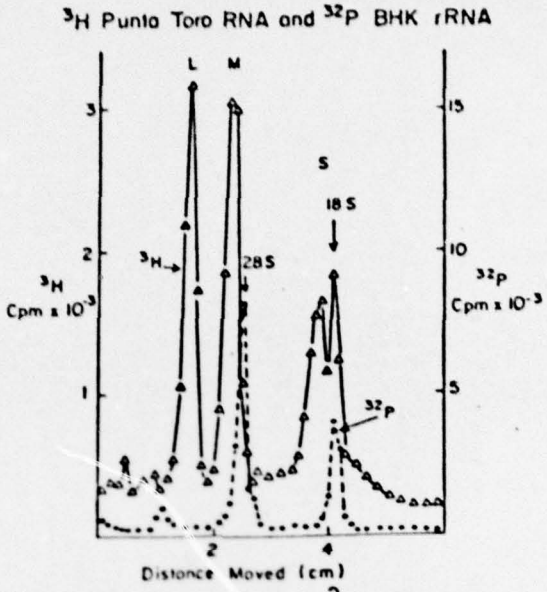

Fig. 9. Coelectrophoresis of ^{3}H KAR viral RNA with ^{32}P BHK 28S and 18S ribosomal RNA species.

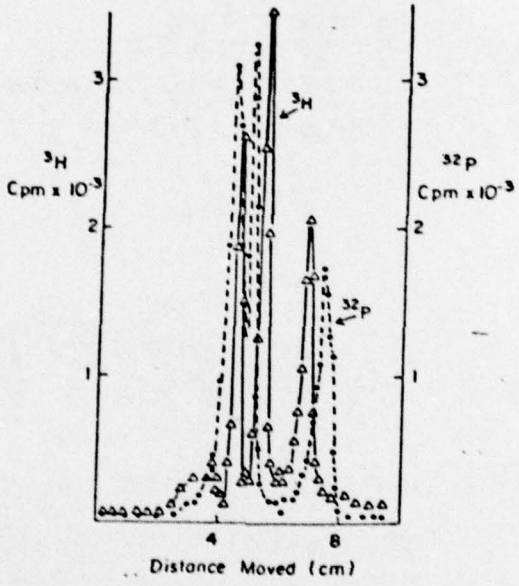

Fig. 10. Coelectrophoresis of ^{3}H KAR viral RNA with ^{32}P SSH viral RNA species.

Viral polypeptides.

Polyacrylamide gel electrophoresis of PT viral polypeptides on 8% polyacrylamide gels indicates that there are 3 major viral polypeptides (Fig. 6). No analyses have been undertaken on the reduced as opposed to non-reduced forms of the viral polypeptides.

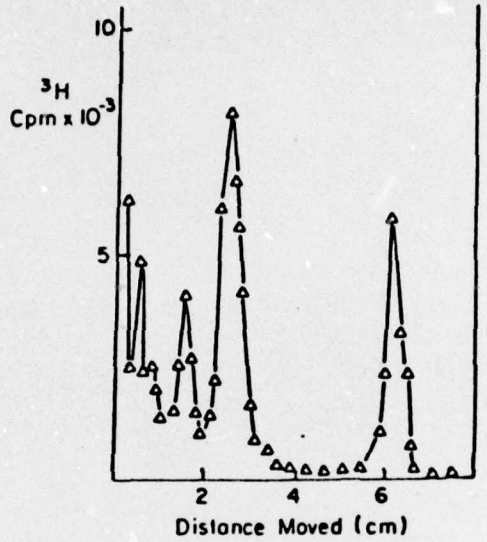

Fig. 11. Polyacrylamide gel electrophoresis of ^{3}H-leucine labeled PT viral polypeptides.

Chagres (CHG) virus.

Viral RNA species.

Sucrose gradient centrifugation of ^{32}P labeled CHG viral RNA extracts have identified 3 major viral RNA species (Fig. 12). No other analyses have been undertaken.

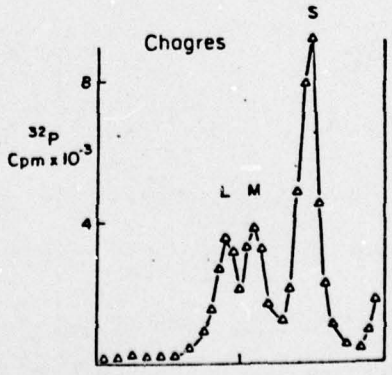

Fig. 12. Sucrose gradient centrifugation of CHG viral RNA species.

Viral polypeptides.

Three major polypeptides have been identified from ^{3}H leucine labeled CHG virus preparations (Fig.13). No further analyses have been performed.

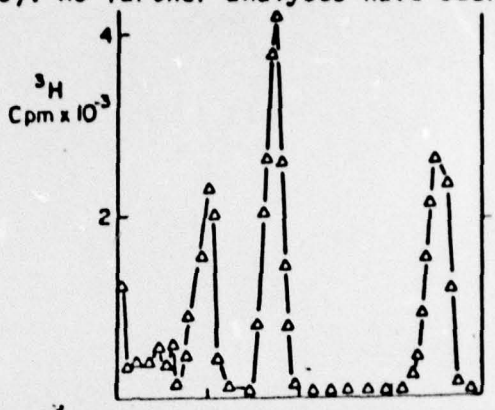

Fig. 13. ^{3}H leucine CHG viral polypeptides resolved by polyacrylamide gel electrophoresis.

Sicilian sandfly fever (SFS) virus.

Viral RNA species.

^{32}Resolution by SDS sucrose gradient centrifugation of ^{32}P labeled SFS viral preparations have identified a large (L), medium (M) and small (S) viral RNA species (Fig. 12). No further analyses have yet been performed.

Viral polypeptides.

^{3}Three major polypeptides have been resolved in ^{3}H leucine labeled SFS virus preparations (Fig. 14). No further analyses have been performed.

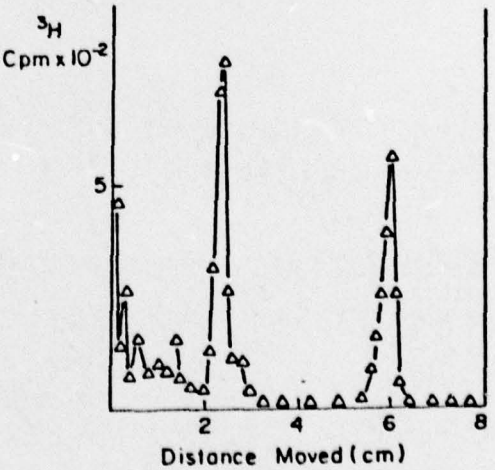

Fig. 14. ^{3}H-leucine SFS viral polypeptides resolved by polyacrylamide gel electrophoresis.

e. Naples sandfly fever virus (SFN), Candiru (CDU), Itaporanga (ITP), and Icoaraci (ICO) viruses.

No analyses of the RNA or virion polypeptides of these viruses have yet been undertaken.

B. Summary of Progress Report.

The analyses undertaken in the initial 6 months of this contract have established that the Phlebotomus fever viruses so far analysed have tripartite RNA genomes. A segmented genome is a prerequisite for genetic reassortment.

The viral polypeptides of some Phlebotomus fever viruses are being investigated in order to eventually determine the RNA segment coding functions. Although the analyses are incomplete at this stage, it should be possible to determine both the location and interrelationships of the major viral polypeptides.

C. Literature cited.

Clewley, J., Gentsch, J., and Bishop, D. H. L., 1977, Three unique viral RNA species of snowshoe hare and La Crosse bunyaviruses. J. Virol. 22:459.

Gentsch, J., and Bishop, D. H. L., 1976, Recombination and complementation between temperature sensitive mutants of the bunyavirus, snowshoe hare virus. J. Virol. 20:351.

Gentsch, J., Bishop, D. H. L., and Obijeski, J. F., 1977a, The virus particle nucleic acids and proteins of four bunyaviruses. J. Gen. Virol. 34:257.

Gentsch, J., Wynne, L. R., Clewly, J. P., Shope, R. E., and Bishop, D. H. L., 1977b, Formation of recombinants between snowshoe hare and La Crosse bunyaviruses. J. Virol. 24:893.

12 Copies	Director (ATTN: SGRD-UWZ-AG) Walter Reed Army Institute of Research Walter Reed Army Medical Center Washington, D.C. 20012
4 Copies	HQDA (SGRD-AJ) Fort Detrick Frederick, Maryland 21701
12 Copies	Defense Documentation Center ATTN: DDC-TCA Cameron Station Alexandria, Virginia 22314
1 Copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, Maryland 20014
1 Copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234