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RIFT VALLEY FEVER VIRAL PROTEINS.(U)
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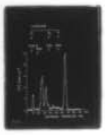

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# Rift Valley Fever Viral Proteins

ROBERT M. RICE,* ROBERT R. ROSATO, GERALD A. EDDY, AND SASHI B. MOHANTY†

United States Army Medical Research Institute of Infectious Diseases

Fort Detrick, Frederick, Maryland 21701

and

†Department of Veterinary Science, University of Maryland

College Park, Maryland 20740



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Abstract

Results of polyacrylamide gel electrophoresis of a Rift Valley fever virus isolate support the tentative classification in Bunyaviridae family.

Rift Valley fever (RVF) is an acute arthropod-borne viral disease of many species, primarily of sheep, cattle and men (1, 2, 5, 8, 9). In animals the disease is characterized by a brief incubation period, a short febrile period, listleness, anorexia and abortion. In addition, focal to diffuse liver necrosis is often present postmortem. In lambs and calves mortality is high while in older animals abortion may be the only clinical sign observed. In humans RVF is characterized by fever, myalgia, headache, weakness, photophobia and icterus. Temporary or permanent blindness can occur due to a macular degeneration (5). However, in a 1977 epizootic of RVF in Egypt, human encephalitic and hemorrhagic deaths occurred. Isolates from the Egyptian epizootic are indistinguishable by cross-neutralization test from isolates of epizootics in South Africa in 1951 and 1975 (C. J. Peters, personal communication).

The interepizootic maintenance cycle of RVF is still unknown (3). Rift Valley fever virus (RVFV) has been shown to be morphologically similar to the Bunyaviruses (9) and thus is a proposed member of the family Bunyaviradae (9, 13).

The purpose of this study was to determine the number of proteins of RVFV and their molecular weights, and compare this information with published data for other members of the Bunyaviradae. The virus used was originally isolated from a sheep during an epizootic of RVF in South Africa in 1951 by injecting infectious serum into mice and subsequently passing 3 times in sheep prior to arrival of the virus in our laboratory (4). An ampoule of the 3rd sheep passage was received from Dr. Jerry S. Walker, Plum Island Animal Disease Laboratory, and was passaged two times in fetal rhesus lung cells, a diploid cell line.

Rift Valley fever virus was absorbed (multiplicity of inoculum 0.001 pfu/cell) to baby hamster kidney-21 (BHK-21, clone 13, #CCL10, American Type Culture Collection, Rockville, Md.) and grown in the presence of 10 μCi/ml $^{3}$H-amino acid mixture (New England Nuclear #NET-250) with Earle's medium 199 1/40 concentration of amino acids (Grand Island Biological Co., Grand Island, N.Y.) 2% heat-inactivated and dialyzed fetal calf serum, 100 units penicillin and 100 μg streptomycin/ml. Tissue culture medium was harvested at maximal cytopathic effect (46-56 h), clarified at 3000 X g in a GSA rotor, and pelleted through a 10-ml layer of 15% Renographin® (E. R. Squibb and Sons, Inc. Princeton, N.J.) at 50,000 X g in a Beckman 21 fixed angle rotor for 2 h. Pellets were resuspended in TNE (0.01 M Tris, 0.15 M NaCl and 0.002 M EDTA, pH 7.4) and layered onto a continuous 15-40% Renographin-TNE gradient centrifuged at 150,000 X g in SW41 rotor for 4 h. The resulting band was harvested, pelleted, resuspended in 100 μl of TNE.

LaCrosse virus (LAC, ATCC#744) was used as a control. It was grown by the same method as RVFV except that it was grown in the presence of 10 μCi [$^{35}$S]methionine/ml and L-methionine-free BME diploid media (GIBCO). It was purified and concentrated as described above. Unlabeled protein molecular weight markers were obtained from Pharmacia Fine Chemicals, Piscataway, N.J. (catalog numbers 17-0445-01 and 17-0446-01).

Viral proteins and standards were resolved by polyacrylamide gel electrophoresis (PAGE), on 8 or 12% discontinuous gels at 3 mA/gel as previously described (7) except dry heat at 100°C for 15-20 min was used to denature the virus prior to electrophoresis. Following

electrophoresis, gels were frozen, sliced into 1-mm sections and placed in scintillation vials with 7 ml of fluid consisting of 150 ml of Liquifluor and 100 ml Protosol (New England Nuclear) per 3 liters of spectral grade toluene and processed as previously described (14). Gels containing unlabeled proteins were fixed and stained (6).

Figure 1 depicts the protein profile of a $^{3}$H-amino acid labeled RVFV preparation. The distance migrated for the 4 proteins L, $G_1$, $G_2$ and of LAC are represented by the top row of arrows and were calculated to be 181,000, 122,000, 41,000 and 27,000 daltons from the nonlabeled protein standards. These values correspond to reported values (10) recorded in Table 1. The second row delineates the distance migrated from protein standards: catalase, phophorylase b, bovine serum albumin, ovalbumin and lactate dehydrogenase subunit.

The majority of the viral protein is present in a split peak (approximately 46mm and 50 mm) and a large single peak (90 mm). Molecular weights for these peaks were calculated to be 70,000, 65,000 and 23,000 daltons and the minor proteins of 12, 25, and 27 mm had estimated molecular weights of 181,000, 125,000 and 87,000 daltons. The values reported here are an average of data from seven gels and agree with values obtained on repeat experiments. The split peak was clearly separated into two components on 12% gels (Fig. 2).

Table 1 compares experimental values for the major proteins of RVFV with reported values (10, 12, Roberson, G., L. H. ElSaid, W. E. Brandt, W. J. M. Dalrymple, and D. H. L. Bishop, submitted to J. Virol., 1979) of other members and proposed members of the Bunyaviradae. The profile of RVFV resembles those of Uukuniemi

(UUK) and Karimabad (KAR), proposed members of the family, more closely than those of the Bunyamwera supergroup, LAC and Bunyamwera (BUN). UUK (11, 12) and KAR (Roberson et al., 1979) are two bunya-like viruses each of which has been reported to have two proteins (75,000 and 65,000 and 62,000 and 50,000 daltons, respectively) corresponding to the split peak in Fig. 1 which travel either as a single peak or as two peaks on PAGE analysis, depending on the conditions.

The 181,000-dalton protein of RVFV corresponds to the reported L protein of LAC and UUK. The RVFV appears to differ from the other Bunya-like viruses by having an additional protein of 125,000 daltons. KAR is reported to have a corresponding protein (Roberson, et al., 1979) which is not consistently present. The other minor peak shown in Fig. 1 (87,000 daltons) was less prominent (Fig. 2) or completely absent in other preparations. Fig. 2 denotes a minor peak at approximately 45 mm which was present inconsistently. These minor peaks may be contaminating cellular protein or possibly viral protein aggregates. Further purification was not always possible because when RVFV was isopycnically banded a second time the virions would disrupt, resulting in purified nucleocapsid (23,000 daltons) shown by electron micrographs and PAGE analysis (R. M. Rice, unpublished data).

The similarities of PAGE analysis of RVF viral proteins with those of other Bunya-like viruses support the tentative classification in the family Bunyaviradae.

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TABLE 1. Major proteins of RVFV, other bunyaviruses and bunya-like viruses

| Virus | Major proteins (daltons) | | | References |
|---|---|---|---|---|
| RVFV | 70,000 | 65,000 | 23,000 | - |
| UUK | 75,000 | 65,000 | 25,000 | 12 |
| KAR | 62,000 | 50,000 | 21,000 | Roberson et al., 1979 |
| LAC | 120,000 | 35,000 | 25,000 | 10 |
| BUN | 125,000 | 30,000 | 22,000 | 10 |

Comparison of major proteins of Bunyamwera virus (BUN), the prototype of the Bunyaviradae, and LaCrosse virus (LAC) member of the California group within the Bunyamwera supergroup, and Uukuniemi virus (UUK) and Karimabad (KAR) possible members of the Bunyaviradae or Bunya-like viruses.

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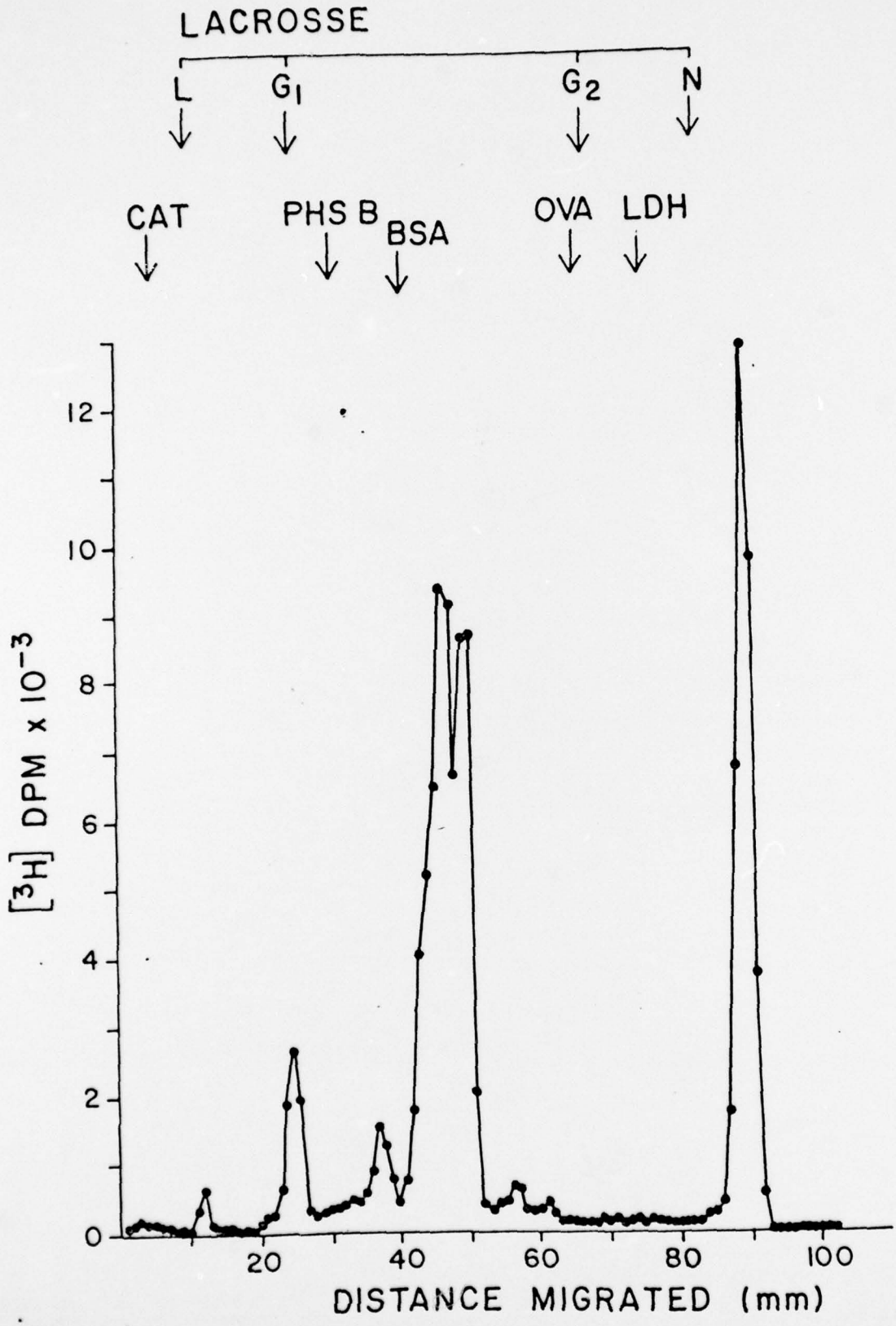
## Figure Legends

FIG. 1. Electrophoresis of tritiated amino acid labeled RVFV on 8% polyacrylamide gels. Standards included $^{35}$S-LaCrosse virus (with L, $G_1$, $G_2$, and N proteins having molecular weights of 180,000, 120,000, 35,000 and 25,000 daltons) (10) and the proteins: catalase (CAT), phosphorylase B (PHS B), bovine serum albumin (BSA), ovalbumin (OVA) and lactate dehydrogenase subunit, (LDH) (with molecular weights of 232,000, 94,000, 67,000, 43,000 and 36,000 daltons, respectively). Other protein standards used in determining the molecular weights of RVFV proteins but not depicted here included catalase subunit, carbonic anhydrase and trypsin inhibition with molecular weights of 60,000, 30,000 and 20,100 daltons. Gels containing the [$^{35}$S]methionine LAC and the protein standards were run on parallel gels.

FIG. 2. Electrophoresis of $^{3}$H-amino acid labeled RVFV on 12% polyacrylamide gels. Standards included $^{35}$S-LaCrosse virus with the $G_1$, $G_2$, and N proteins having molecular weights of 120,000, 35,000 and 25,000 daltons (10) and proteins phosphorylase B(PHS B), bovine serum albumin (BSA), ovalbumin (OVA), and carbonic anhydrase (CBA), having molecular weights of 94,000, 67,000, 43,000 and 30,000 daltons. Gels containing the [$^{35}$S]methionine LAC virus and protein standards were run on parallel gels.


LACROSSE
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G1
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CAT
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BSA
OVA
LDH
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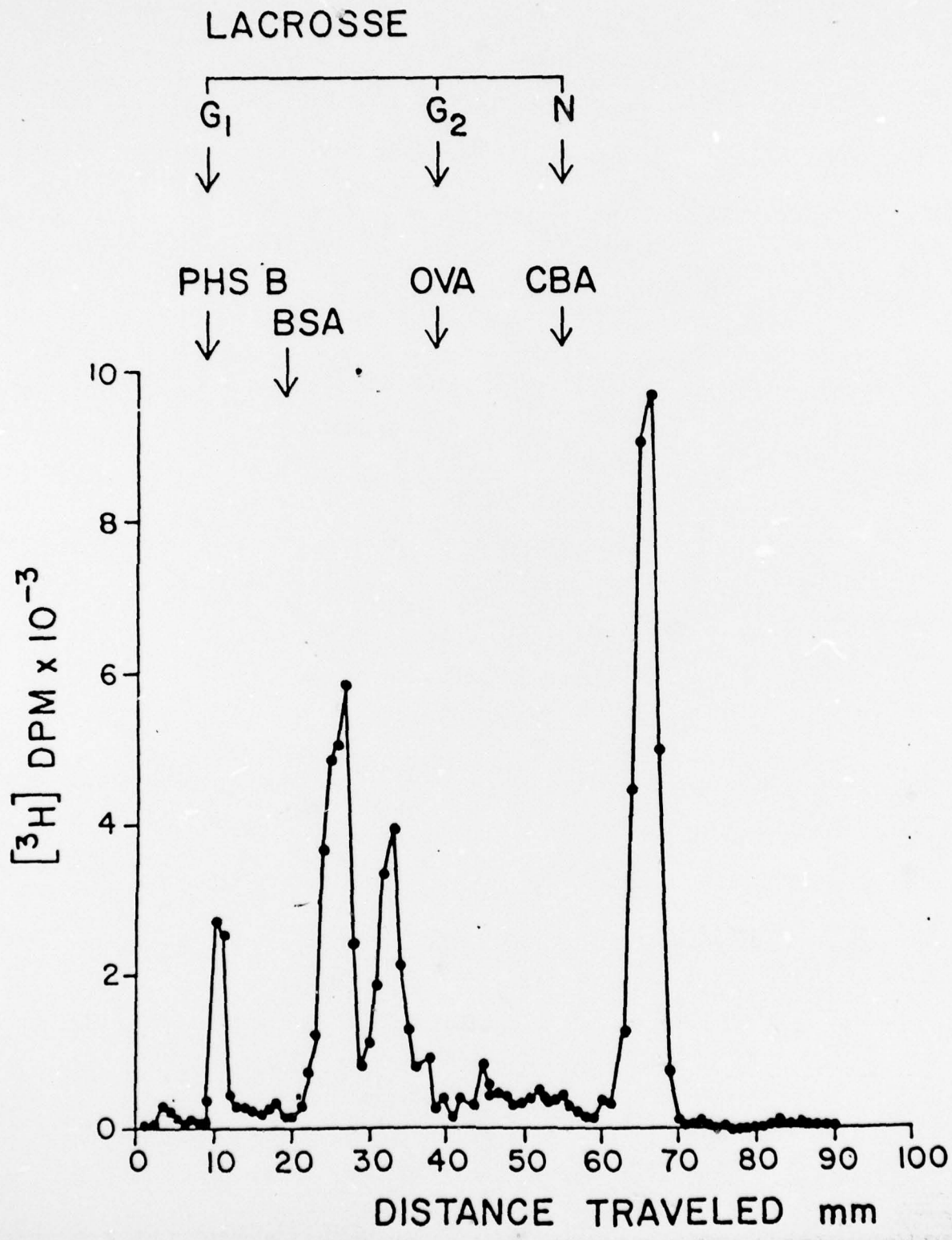
DISTANCE MIGRATED (mm)

Fig. 1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DISTANCE TRAVELED mm

Fig 2