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AD 432423

TECHNICAL MANUSCRIPT 129

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FEBRUARY 1964

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

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PROTECTION OF MICE AND LAMBS AGAINST PANTROPIC RIFT VALLEY FEVER VIRUS WITH IMMUNE SERUM

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Project 1C5223[illegible]A059

February 1964

Portions of the work reported here were performed under Projects 1C022301A067 and 4B11-02-065, "Viral and Rickettsial Agent Research," Task -02, "Viral and Rickettsial Agent Laboratory Research." Expenditure order was 2077. This material was originally submitted as manuscript 5323.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Mr. Howard Waltz, Mr. Gerald DeBree, and Mr. Charles Biddlecome.

ABSTRACT

Immune serum was used prophylactically and therapeutically in mice and lambs infected with Rift Valley fever virus. One-tenth milliliter of immune serum was effective in protecting mice against challenge with Rift Valley fever virus for a period of two weeks but not three weeks. A high percentage of mice receiving immune serum within 15 hours after the inoculation of 10^4 $MIPLD_{50}$ of virus were protected. When serotherapy was administered to mice 11 to 25 hours after challenge, pathogenesis was altered so that the usual pantropic nature of the virus was masked and a neurotropic propensity appeared. This was demonstrated by delayed deaths, symptoms involving the central nervous system, and high titers of virus in the brain of the mice. Serotherapy was also effective in one- to three-day-old lambs infected with Rift Valley fever virus. Protection was demonstrated when immune serum was administered after the appearance of viremias and clinical signs of illness. All surviving lambs that received serotherapy were immune to a challenge infection approximately 30 days later.

I. INTRODUCTION

It has long been established that under certain circumstances serum prophylaxis and serum therapy can effectively alter the disease-producing potential of certain viruses. Stefanapoulo and Nagano[1] studied passive immunity and serotherapy in Rift Valley fever infections in mice and showed that immune serum given as early as 15 days prior to, or as late as 36 hours after, virus inoculation would fully protect mice from otherwise lethal doses of virus.

The experiments presented in this report confirm the results of Stefanapoulo and Nagano and show evidence of an alteration of viral properties and distribution in mouse tissues as a result of the administration of antiserum. Our studies also included a study of the effectiveness of serotherapy in newborn lambs infected with Rift Valley fever virus.

II. MATERIALS AND METHODS

A. VIRUS

The Rift Valley fever (RVF) virus employed was the "van Wyk" strain isolated in 1951 by Kaschula.[2] In our laboratory, pools of virus were prepared from the serum of young lambs that had been inoculated by the intraperitoneal route with 300 mouse intraperitoneal LD_{50} doses ($MIPLD_{50}$) of virus.* Infected lambs were exsanguinated when they became moribund (approximately 46 hours after virus inoculation). The collected serum had a virus titer of $10^{7.0}$ $MIPLD_{50}$ per milliliter. In the following experiments, challenge doses ranging from $10^{3.5}$ to $10^{4.0}$ $MIPLD_{50}$ were administered by the intraperitoneal route. Mice received 0.1 milliliter and lambs were inoculated with 1.0 milliliter.

B. MICE

Mice used in these experiments weighed 10 to 14 grams (21 to 28 days old) and were from the Swiss-Webster strain. No attempts were made to select males or females.

C. LAMBS

One- to three-day-old lambs of mixed breeds were obtained locally.

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* In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

D. IMMUNE SERUM

Immune serum was obtained from calves that had been inoculated with viable Rift Valley fever virus. The calves were bled at about 21 days following infection and the serum was stored in a dry ice chest. In serum neutralization tests performed according to Easterday et al,[3] the calf serum was capable of neutralizing all dilutions of virus including mixtures of equal volumes of serum and undiluted virus (neutralization index, 10^{+0}). Aliquots of serum were thawed and inactivated at 56°C for 30 minutes prior to use. In all experiments in which mice were used, the undiluted serum was inoculated by the intraperitoneal route. In lambs, 10 to 30 milliliters of serum were inoculated by the intraperitoneal and intravenous routes simultaneously or by the intraperitoneal route alone.

E. VIRUS TITRATIONS

Titrations were performed with decimal dilutions of virus suspensions in beef-heart infusion broth (Difco). One-tenth milliliter of each dilution was inoculated intraperitoneally into each of five mice. The median lethal doses were calculated by the method of Reed and Muench.[4]

III. RESULTS

A. PASSIVE IMMUNITY IN MICE

Initial studies showed that when antiserum was administered prior to virus inoculation mice were protected for a two-week period (Table I). Serum injection that preceded virus inoculation by a three-week period showed no protective effects. Also, under the prescribed conditions of this experiment, 0.1 milliliter of serum was as effective as 0.5 milliliter.

B. SEROTHERAPY IN MICE

Table II presents results from an experiment in which separate groups of ten mice each were treated with 0.1 milliliter of immune serum at successive hourly intervals following virus inoculation. These results show that the therapeutic activity of immune serum can be demonstrated by increased survival rates and survival times. Most mice treated with serum within 15 hours after virus inoculation were fully protected. There was a delay of 2.8 to 5 days in the mean day of death in mice that received serum from 11 to 30 hours after the virus. When serum was injected 31 to 44 hours after infection, almost all mice died within four days.

TABLE I. DURATION OF PASSIVE IMMUNITY TO RIFT VALLEY FEVER VIRUS IN MICE

Time Of Challenge[a] (days after antiserum)	Number Of Mice Surviving[b] (dose of antiserum)	
	0.1 ml	0.5 ml
7	9	10
14	7	7
21	0	0

a. Mice were challenged with 10^4 $MIPLD_{50}$ of virus.
b. Ten mice treated in each group.

TABLE II. SEROTHERAPY OF MICE INOCULATED WITH 10^4 $MIPLD_{50}$ OF RIFT VALLFY FEVER VIRUS

Time Of Serum[a] Inoculation, hr	Per Cent Dying 1-5 days	Per Cent Dying 6-10 days	Mean Day Of Death	Per Cent Survival
1-5	14	4	4.3	82
6-10	16	4	4.1	80
11-15	8	14	6.1	78
16-20	4	58	7.0	38
21-25	14	72	6.5	14
26-30	48	36	4.8	16
31-35	68	24	3.8	8
36-40	90	8	2.8	2
41-44	97.5	0	2.4	2.5
control	100	0	2.0	0

a. Serum treatment groups of five consecutive hourly intervals are combined to simplify the presentation of data. At each hourly interval, ten mice were tested.

It was noted that almost all mice that died after the fifth day showed signs of paralysis prior to death, suggesting that prolonged survival resulted in virus activity in the central nervous system. This delayed CNS response was more closely investigated in another experiment in which the virus concentration in mouse livers and brains were determined daily. A group of mice was inoculated with 10^4 $MIPLD_{50}$ of RVF virus, and 0.1 milliliter of immune serum per mouse was administered to various groups of from 50 to 80 mice at 1, 10, 16, 22, 23, 24, 31, and 33 hours following virus inoculation. Each day for ten days the mice that had died in each group were counted and discarded. The survivors in each group were divided into those showing no symptoms, those showing typical generalized symptoms, and those paralyzed. Four mice were sacrificed daily for virus studies and were selected to approximate the proportion of normal, ill, and paralyzed mice in the group at the time of sacrifice. The brains and livers from each group were titrated in mice to determine virus concentrations.

Observation of each group showed that mice surviving longer than five days exhibited paralysis rather than a generalized illness prior to death (Figure 1). Deaths occurring earlier than Day 5 were preceded by generalized illness and no paralysis. The daily brain and liver titers in each group (Figure 2) showed that the titers during the first few days were higher in the livers than in the brains, but the reverse was true during the later days. On Days 8 to 10, in nearly all cases, there was no detectable virus in the liver and relatively high titers in the brains.

To rule out the possibility that serotherapy induced a latent virus infection that might become manifest after passive immunity waned, the following experiment was performed. Mice were inoculated with 10^3 $MIPLD_{50}$ of RVF virus. Fifty mice were given 0.1 milliliter of immune serum at 10 hours and another 50 received the same treatment at 15 hours. Livers from mice that died within 90 days and the remainder, which were sacrificed at 90 days, were examined for virus. No virus was detected in any of these mice after the seventh postinoculation day.

C. SEROTHERAPY IN LAMBS

When immune serum was administered to virus-inoculated lambs before a viremia developed, no evidence of infection followed (Table III). Five of seven lambs (Nos. 24, 25, 32, 33, 36, 41, 42) that had viremias at the time of serum therapy survived. These five lambs had viremias of from $10^{4.2}$ to $10^{7.8}$ $MIPLD_{50}$ of RVF virus per milliliter of blood prior to serotherapy. Three lambs (Nos. 32, 33, 42) that survived were exhibiting signs typical of Rift Valley fever, as described by Easterday,[5] prior to serum therapy. Both of the lambs (Nos. 36 and 41) that died were prostrate at the time serum was administered.

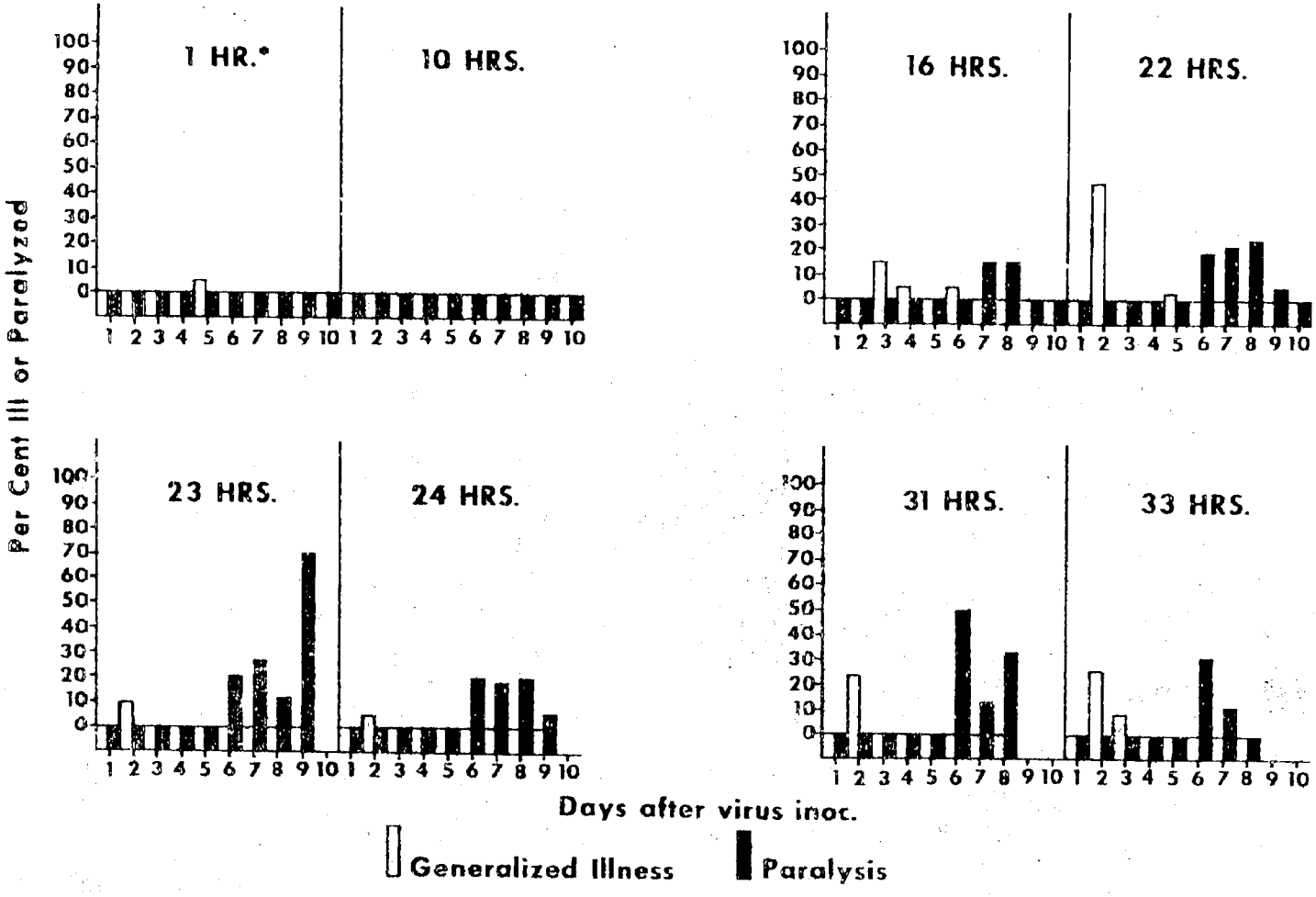

Figure 1. Distribution of Symptoms in Mice Receiving Immune Serum after Inoculation with Rift Valley Fever Virus.

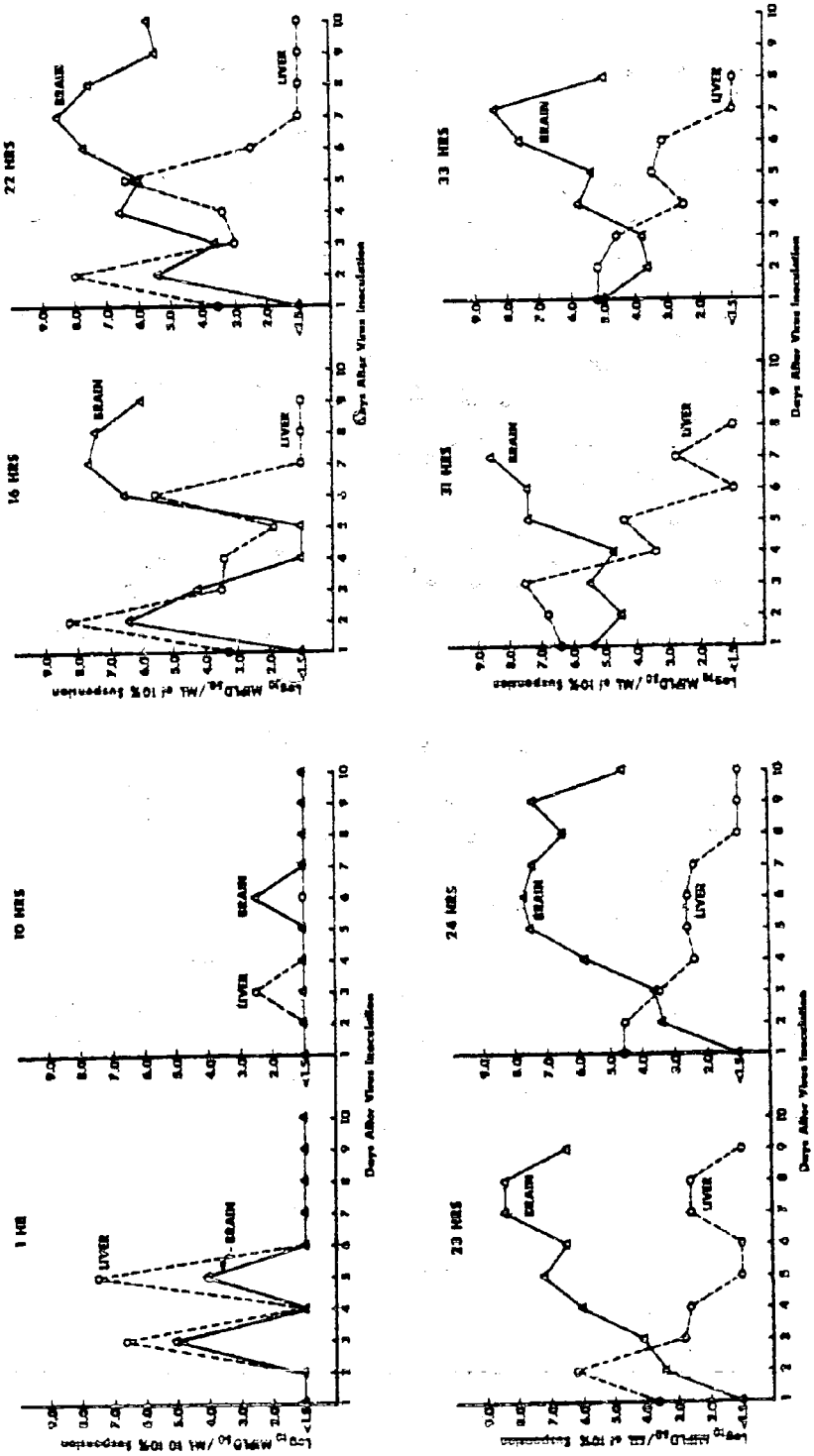

Figure 2. Titers of Rift Valley Fever Virus in Brains and Livers of Mice after Treatment with Immune Serum.

TABLE III. SEROTHERAPY OF LAMBS INFECTED WITH RIFT VALLEY FEVER VIRUS

Lamb No.	Virus Dose Log $MIPLD_{50}$	Viremia Log $MIPLD_{50}$/ml	Time of Antiserum Injection[a]	Symptoms Prior to Serum Injection	Time of Death[b]
19	2.5	neg (24), neg (45)	4[b]	none	S[c]
20	2.5	neg (24), neg (45)	20	none	S
18	2.5	3.7 (24), 8.3 (45)	-	-	68
21	3.5	neg (24), neg (45)	6	none	S
22	3.5	neg (24), neg (45)	6	none[d]	S
24	3.5	4.8 (24), neg (45)	24	none[d]	S
25	3.5	4.2 (24), neg (45)	24	none[d]	S
23	3.5	neg (24), 10.4 (45)	-	-	47
26	3.5	4.8 (24), 9.7 (45)	-	-	47
27	3.5	5.0 (24), 9.0 (45)	-	-	45
28	3.5	4.5 (24), 9.0 (45)	-	-	47
29	3.5	4.3 (24), 9.8 (45)	-	-	47
32	4.0	5.8 (24), 6.4 (29)	29	yes	S
33	4.0	6.5 (24), 6.9 (29)	29	yes	S
36	4.0	5.9 (24), 7.6 (46)	46	yes	50
41	4.0	8.6 (36), 8.3 (43)	36	yes	44
42	4.0	7.6 (36), neg (60)	39	yes	S
31	4.0	8.9 (24)	-	-	46
34	4.0	9.4 (24)	-	-	46
40	4.0	9.5 (36), 10.0 (43)	-	-	44

a. Lamb 19 received 10 ml intravenously (IV) and 20 ml intraperitoneally (IP).
Lamb 20 received 10 ml IV and 10 ml IP.
Lambs 21 and 24 received 10 ml IP.
All others received 5 ml IV and 5 ml IP.

b. Hour after virus inoculation.

c. Survived.

d. Febrile response four hours after serum inoculation.

Twenty-five to 41 days after the initial virus dose, all surviving lambs were challenged with 10^5 $MIPLD_{50}$ of RVF virus in an inoculum that was divided equally between the intraperitoneal and subcutaneous routes. All lambs survived challenge without exhibiting signs of disease or detectable viremias. A control lamb of approximately the same age died on the fifth day after challenge.

IV. DISCUSSION

Protection of mice inoculated with Rift Valley fever virus with a specific antiserum was demonstrated by survival and delayed mortality. Mortality was delayed when serum was administered only a few hours before the normal time of death. Serotherapy, administered as late as 15 hours after inoculation with a dose of virus that killed most of the control mice within 48 hours, resulted in almost complete protection.

An alteration in the pathogenesis of the virus in mice was most obvious in those animals treated with immune serum late in the incubation period (11 to 25 hours). Deaths in this group were delayed and invariably were preceded by paralysis, which was not observed in untreated mice. Further investigations showed that during this period virus was found in greater concentration in the brain than in the liver. Evidently serotherapy was more effective in controlling the visceral pathogenesis of the virus than it was in suppressing the multiplication of the virus in the brain. These experiments have shown that a virus, viscerotropic in mice under ordinary conditions, had a propensity for the central nervous system that became apparent when animals were treated with antiserum. Perhaps an explanation of these results may be found in the work by Fox,[6] who showed that yellow fever virus antibody in the brain did not reflect the antibody concentrations in the serum. The existence of a barrier between the vascular and central nervous systems may have effectively reduced the transfer of humoral antibody while allowing invasion by a few virus particles. Because the virus is a replicating entity, and the antibody is not, it would be conceivable that infection could be established in the brain while high levels of antibody were found in the serum. This hypothesis is supported by the studies of Hsieh *et al*,[7] who showed that treatment of mice infected with Japanese B encephalitis virus with immune serum was more effective when the animals were exposed to carbon dioxide. These authors postulated that the exposure to carbon dioxide facilitated the passage of passively administered antibody from the serum to the central nervous system, thereby conferring greater protection.

The possibility that the virus in the central nervous system underwent a genetic alteration because of a selective action by the immune serum was investigated by subinoculation of brain tissues. The virus from the brains of serum-treated mice showed no evidence of any change when further passed in mice. All mice died within four days without showing signs of paralysis.

Traub[8] showed that passive immunity suppressed Eastern equine encephalomyelitis virus in mice and that the treated mice could become ill and die several weeks later. This effect was apparently brought about by the virus remaining latent until immunity waned to the point of ineffectiveness. A similar experiment described in the present paper gave no indication that this phenomenon had occurred with Rift Valley fever virus.

The serotherapy studies conducted in lambs showed that serum treatment was highly effective in protecting the host from fatal infections with Rift Valley fever virus. Treatment was found to be effective after viremias had been clearly established, and in a few cases complete recovery resulted when serum was administered after clinical signs of the disease were evident. Many instances of effective serotherapy for viral diseases have been recorded[1,9-12] when serum was inoculated prior to the appearance of the signs of the disease, but in only a few cases[7,13-15] has serum shown a protective effect after clinical signs have appeared.

The effectiveness of serotherapy after the onset of clinical signs in lambs suggests a possible practical application in the control of Rift Valley fever. The possibility of using live virus in conjunction with immune serum to produce passive-active immunity is also indicated.

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