AD

TECHNICAL MANUSCRIPT 364

INACTIVATION OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS BY GAMMA RADIATION

Morton Reitman
Henry R. Tribble, Jr.

APRIL 1967

STATEMENT #2 UNCLASSIFIED

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Dept of the Army Ft Detrick Attn: Tech Release Br - Tech Info Div.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 364

INACTIVATION OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS BY GAMMA RADIATION

Morton Reitman

Henry R. Tribble, Jr.

Medical Investigation Division
MEDICAL SCIENCES LABORATORY
and
Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORY

Project 1B622401A072

April 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

Exposure of Venezuelan equine encephalomyelitis (VEE) virus at -70 C to 6 x 10^6 r gamma radiation (Co^{60}) resulted in loss of lethality for young adult mice and guinea pigs and loss of capacity to produce plaques or cytopathic effects in tissue culture. The suckling mouse was more sensitive for detecting live virus in irradiated suspensions than the adult mouse or guinea pig. Live virus was demonstrable in preparations exposed to 6 x 10^6 r but not in suspensions exposed to 8 x 10^6 r or more. The rate of inactivation of VEE virus by gamma radiation was an exponential function of the dosage.

I. INTRODUCTION

It has been reported that gamma rays inactivate the viruses of poliomyelitis, St. Louis encephalitis, western equine encephalitis, vaccinia,[1] influenza, and mumps.[2] The results presented suggest that this method of inactivation destroys the capacity of the virus to produce infectivity in animals but does not alter the antigenicity unless large doses of radiation are employed. These studies prompted our investigation on inactivation of Venezuelan equine encephalomyelitis (VEE) virus by exposure to gamma rays.

II. MATERIALS AND METHODS

A. VIRUS

The Trinidad strain of VEE was used for all experiments. Virus was propagated in monolayers of mouse fibroblast strain L cells or in a Maitland-type culture of chick embryo tissue in a chemically defined medium.[3] Supernatants were harvested and clarified by centrifugation; portions were stored in sealed ampules or rubber-stoppered bottles at -70 C.

B. RADIATION

Virus suspensions were radiated at the National Bureau of Standards, using a 50,000-curie cobalt 60 source emitting radiation at a rate of approximately 7.5×10^6 r per hour. Cobalt glass dosimetry was used to measure the exact dosage delivered. Suspensions were irradiated in glass containers sealed in tin cans in a Dewar flask and inserted into a carrier (Fig. 1). The samples were kept frozen with dry ice during exposure to minimize the indirect lethal action of free radicals.[4] After exposure, samples were stored at -70 C until tested for presence of active virus. Control samples of virus were exposed to identical conditions but were not irradiated.

C. TITRATION OF VIRUS SUSPENSION

Survival curves of irradiated virus were determined by intracerebral (IC) inoculation of 0.03 ml in 10- to 14-g Swiss mice and by plaque assay and capacity to induce cytopathic effects (cpe) in mouse fibroblast strain L cells. Samples of irradiated virus that were nonlethal for adult mice were inoculated into 250- to 350-g guinea pigs. Each of 15 guinea pigs was administered 0.25 ml of an undiluted or a 10^{-1}, 10^{-2}, 10^{-3}, or 10^{-4} dilution of virus. No deaths occurred.

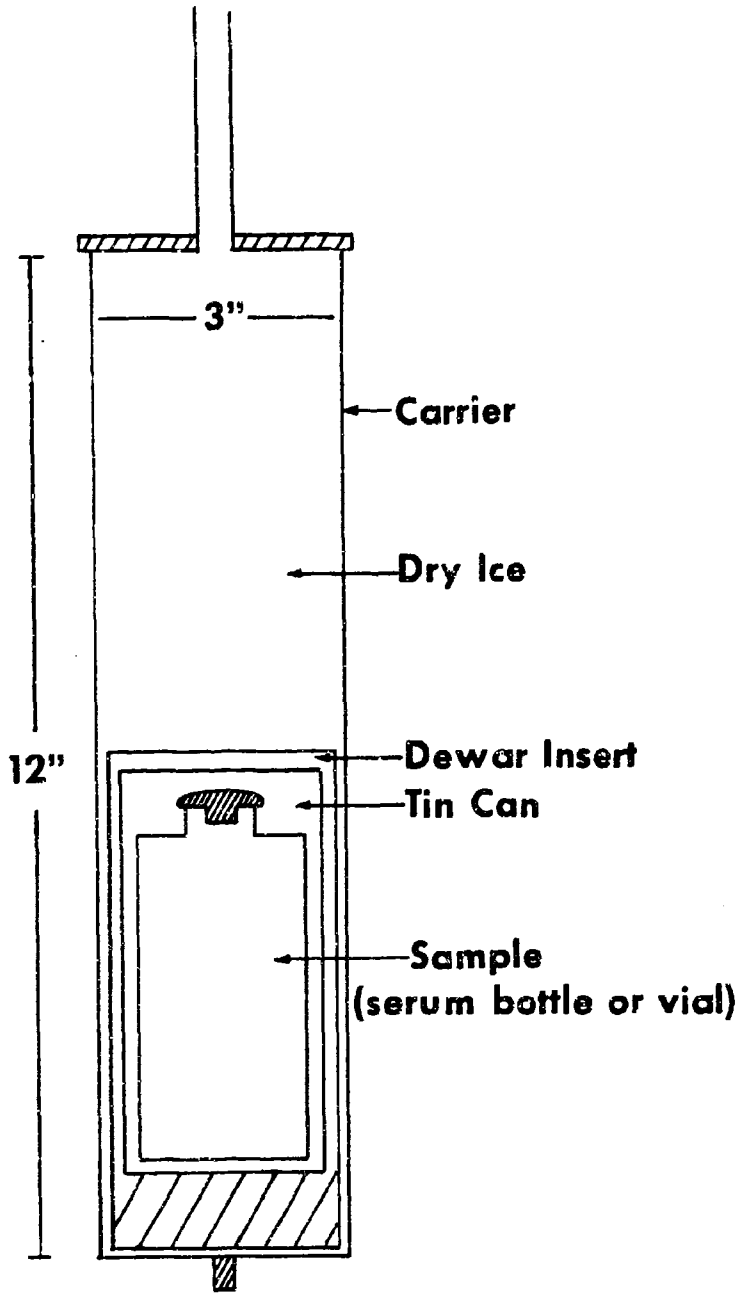

Figure 1. Container Arrangment for Irradiating Samples.

On the basis of data obtained, guinea pigs (250 to 350 g) were inoculated with selected samples of irradiated VEE virus. These samples ranged from those exposed to doses of radiation that failed to inactivate all viable virus to those that were nonlethal to adult mice and guinea pigs. The animals received either one or two inoculations given at an interval of 1 week; dosages are given in Table 3. Five animals were bled 3 weeks postinoculation by intracardial puncture and the level of serum-neutralizing and hemagglutination-inhibiting antibodies was determined. The resistance of the guinea pigs to challenge with virulent homologous virus was determined. A dose of 2.5×10^5 mouse IC lethal doses_{50} (MICLD_{50}) was administered to each animal. Mice were utilized in similar tests but no serological assays were performed.

Virus suspensions were titrated in suckling mice by inoculation of 0.03 ml via the IC and 0.03 ml via the intraperitoneal (IP) route in the same animal. The brains of mice that died 24 or more hours after inoculation were harvested, ground in a mortar, and suspended in beef heart infusion broth. The presence of VEE virus was confirmed by neutralization with VEE antiserum in adult mice.

D. SERUM NEUTRALIZATION TEST

The serum neutralization (SN) test reported by Smith et al.[5] was employed, utilizing male mice only.

E. HEMAGGLUTINATION INHIBITION TEST

Guinea pig sera were tested by the microtiter technique using a VEE hemagglutinin antigen inactivated with beta-propiolactone.

III. RESULTS

Figure 2 shows the effect of various doses of gamma radiation on the survival of VEE virus as determined by assay in adult mice. The median lethal dose (LD_{50}) values, calculated according to the Reed and Muench formula,[6] are plotted on a logarithmic scale as a function of radiation dose. The results show that the rate of inactivation is expressed as an approximately straight line, indicating that the surviving fraction is an exponential function of the dose. Extension of the line indicates that complete loss of mouse lethality could be expected to occur at a dose level of 7.4×10^6 r. However, deaths did not occur when young adult mice were inoculated with virus that had been exposed to radiation doses of 6×10^6 r or greater (Table 1). Similar results were obtained with guinea pigs (Table 2). The capacity to produce plaques or cpe was lost in suspensions exposed to 6×10^6 r or greater.

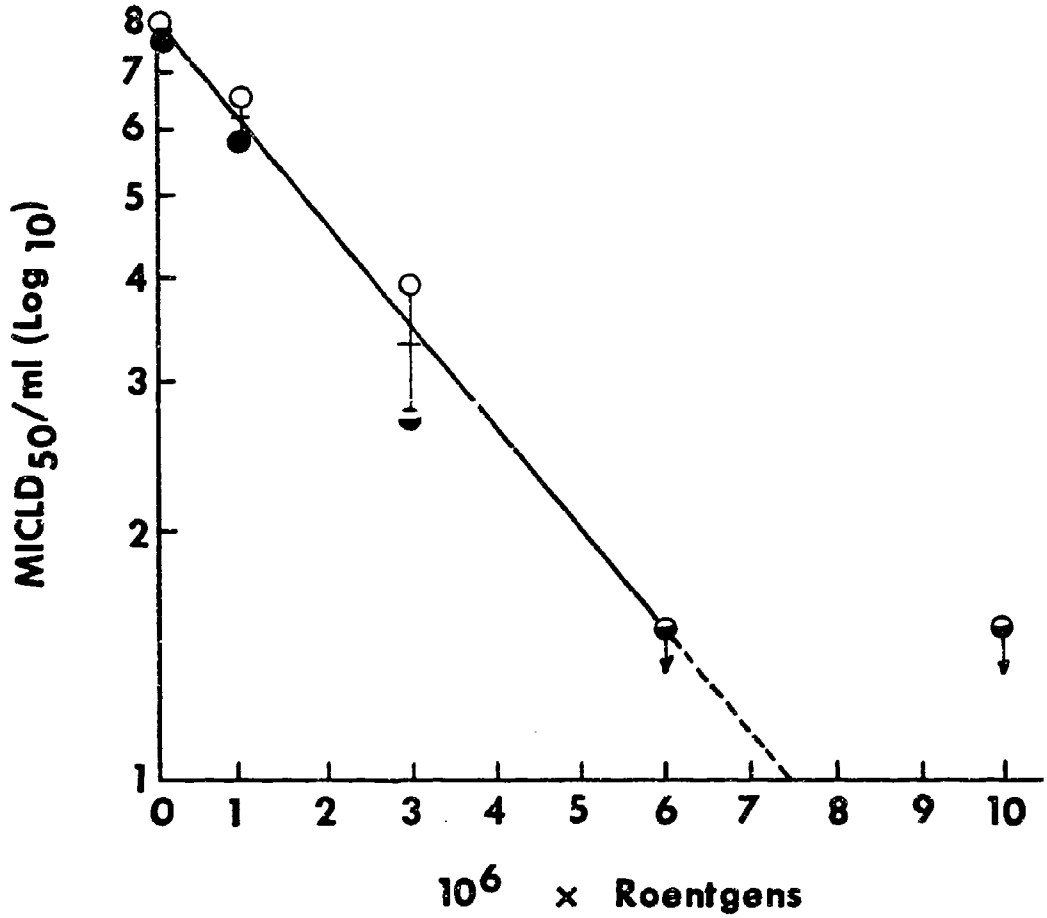

Figure 2. Survival Curve of VEE Virus Obtained by Irradiation with Gamma Rays, Average of Two Experiments.

The results of the assays in suckling mice and the hemagglutination inhibition (HI) and SN tests from a typical experiment are shown in Table 2. The test with suckling mice indicated the presence of live virus in the suspensions irradiated with 5 x 10^6 r and 6 x 10^6 r, but not in those suspensions exposed to larger doses. The virus exposed to 5 x 10^6 r was lethal for 20% of guinea pigs but not for adult mice. High SN indices were obtained in all guinea pigs administered two inoculations of virus that had been exposed to radiations ranging from 5 x 10^6 to 10 x 10^6 r. A significantly lower SN antibody response was obtained in guinea pigs inoculated with virus exposed to 16 x 10^6 r. Assuming that virulence for suckling mice is correlated with the amount of live virus present in the irradiated suspensions, the lowest HI antibody responses were obtained with the preparation containing the greatest amount of live virus. The protective potency determined by antigen extinction assay is expressed here as the effective dose (ED_{50}), the volume of undiluted irradiated virus suspension inoculated per dose that protected 50% of the test animals. The ED_{50} for mice was the same for preparations exposed to radiation doses ranging from 5 x 10^6 r to 8 x 10^6 r but was greater at higher radiation levels.

Table 3 lists the SN antibody response and the ED_{50} in milliliters obtained in guinea pigs that received either one or two inoculations of irradiated virus. The primary antibody response was low in the animals that received only one inoculation. The second inoculation caused an increase in antibody in 50% of the animals. It should be noted that Tables 2 and 3 list the results obtained by inoculation of two different virus preparations and that the guinea pigs listed in Table 2 received 0.25 ml more virus than those listed in Table 3.

TABLE 1. INACTIVATION OF VEE VIRUS BY GAMMA RADIATION AS MEASURED BY PLAQUE FORMATION, cpe, AND MOUSE LETHALITY[a]

Radiation Dose, 10^6 r	$MICLD_{50}$	pfu[b]	cpe[b]
0	10.0	8.3	8.3
1	7.6	6.0	7.5
3	4.1	2.8	3.5
6	<1.5	<1.0	<1.0
10	<1.5	<1.0	<1.0

a. Experiment HV-1, L cell culture. $MICLD_{50}$/ml 10.0 prior to radiation.
Log base 10.
b. Performed by Dr. Henry J. Hearn, Jr.

TABLE 2. ANTIGENICITY AND INFECTIVITY OF IRRADIATED VEE VIRUS[a]

Radiation Dose, 10^6 r	Suckling mice		Adult Mice 10 to 14 g		Guinea pigs	Guinea Pig Serum[b]	
	Dead Total	Live Virus	$IPLD_{50}$/ml, log base 10	ED_{50},[c] ml	$IPLD_{50}$/ml, log base 10	HI	SN Index
5	21/25	+	<0.6	0.003	<0.6[d]	121[e]	$>5 \times 10^6$
6	3/25	+	<0.6	0.003	<0.6	643	$>5 \times 10^6$
8	0/25	-	<0.6	0.003	<0.6	845	$>5 \times 10^6$
10	0/25	-	<0.6	0.04	<0.6	368	8×10^3 to $>5 \times 10^6$
16	0/25	-	<0.6	0.04	<0.6	453	1.6×10^2 to 5×10^3

a. Experiment MR-27. Maitland-type culture. $MICLD_{50}$/ml 9.9, $MIPLD_{50}$/ml 9.6 prior to radiation.
b. Received 0.75 ml total inoculum in two inoculations.
c. Effective dose, based on 0.5 ml total inoculum in two inoculations.
d. Three of 15 died in group that received undiluted treated virus.
e. Geometric mean of HI titers; reciprocal of dilution.

TABLE 3. ANTIBODY RESPONSE OF GUINEA PIGS TO INOCULATION WITH IRRADIATED VEE VIRUS[a]

Radiation Dose, 10^6 r	ED_{50}, ml		SN Index	
	One Inoculation[b]	Two Inoculations[c]	One Inoculation[b]	Two Inoculations[c]
6	0.166	0.008	20	20
			39	10,000
			390	16,000
			0	>63,000
			0	10
8	0.33	0.008	10	>63,000
			16	20
			10	2,000
			10	20
			-	10
10	>0.5	0.008	2	100
			0	15
			2	31
			0	-
			3	-

a. Experiment MR-25. Maitland-type culture. $MICLD_{50}$/ml 9.0 prior to radiation.
b. 0.5 ml.
c. 0.25 ml x 2.

IV. DISCUSSION

The results of tests for inactivation of VEE virus by measuring the capacity of the virus to produce cpe, plaques, or death of adult mice and guinea pigs suggest that active virus was not present in suspensions exposed to 6×10^6 r of gamma radiation. However, live virus was detected in this preparation by inoculation of suckling mice by both the IC and IP routes. Suckling mice have been reported to be considerably more sensitive to infection with VEE virus than either hamster kidney or monkey kidney tissue cells.[7] It is apparent from our experiments that the suckling mouse is more sensitive to the lethal action of irradiated VEE virus than either the adult mouse or guinea pig.

The use of ionizing radiation to inactivate virus presents the difficult problem of determining the absence of live virus in irradiated preparations because the survival curve of irradiated viruses is exponential.[1]

The high levels of SN and HI antibodies obtained in the guinea pig (Table 2) might be due either to an inapparent infection with live virus or to the stimulation of antibody production upon introduction of additional inactivated virus in the second inoculation. The latter appears to be the more logical explanation. The data listed in Table 3 show that one inoculation was not sufficient to induce significant antibody titers; a second inoculation resulted in a sharp rise in the level of neutralizing antibody. These results also support the conclusion that live virus was not present in sufficient quantity to produce infection, because it would be expected that significant levels of antibody would have resulted from a single inoculation.

The reduced antibody response that occurred at radiation doses of 10×10^6 r or higher may be attributable to breakdown of antigenic material; this phenomenon has been reported to occur with the viruses of influenza[7] and vaccinia.[8]

V. SUMMARY

A strain of VEE virus was inactivated by exposure to a radiation dose of 8×10^6 r of gamma radiation. The suckling mouse was more sensitive for detecting live virus in irradiated suspensions than the adult mouse or guinea pig. The rate of inactivation of VEE virus by gamma radiation was an exponential function of the dosage.

LITERATURE CITED

1. Jordan, R.T.; Kempe, L.L. 1956. Inactivation of some animal viruses with gamma radiation from cobalt 60. Proc. Soc. Exp. Biol. Med. 91:212-215.

2. Polley, J.R. 1962. The use of gamma radiation for the preparation of virus vaccines. Can. J. Microbiol. 8:455-459.

3. Nagle, S.C.; Tribble, H.R.; Anderson, R.E.; Gary, N.D. 1963. A chemically defined medium for growth of animal cells in suspension. Proc. Soc. Exp. Biol. Med. 112:340-344.

4. Sykes, G. 1965. Disinfection and sterilization. 2nd ed. E & F Spon Ltd., London. 486 p.

5. Smith, D.G.; Mamay, H.K.; Marshall, R.G.; Wagner, J.C. 1956. Venezuelan equine encephalomyelitis: Laboratory aspects of fourteen human cases following vaccination and attempts to isolate the virus from the vaccine. Amer. J. Hyg. 63:150-164.

6. Reed, L.J.; Muench, H. 1938. A simple method of estimating fifty per cent endpoints. Amer. J. Hyg. 27:493-497.

7. Kudin, W.D.; Liu, C.; Rodina, P. 1966. Pathogenesis of Venezuelan equine encephalomyelitis virus: I. Infection in suckling mice. J. Immunol. 96:39-48.

8. Polley, J.R. 1961. Factors influencing inactivation of infectivity and hemagglutinin of influenza virus by gamma radiation. Can. J. Microbiol. 7:535-541.

9. Kaplan, C. 1960. The antigenicity of γ-irradiated vaccinia virus. J. Hyg. 58:391-398.

Unclassified
Security Classification

DOCUMENT CONTROL DATA · R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Department of the Army Fort Detrick, Frederick, Maryland, 21701	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP

3. REPORT TITLE
INACTIVATION OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS BY GAMMA RADIATION

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (Last name, first name, initial)
Reitman, Morton (NMI)
Tribble, Henry R., Jr.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
April 1967	14	9

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1B622401A072	Technical Manuscript 364
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10. AVAILABILITY/LIMITATION NOTICES
Qualified requesters may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY Department of the Army Fort Detrick, Frederick, Maryland, 21701

13. ABSTRACT

Exposure of Venezuelan equine encephalomyelitis (VEE) virus at -70 C to 6 x 10^6 r gamma radiation (Co^{60}) resulted in loss of lethality for young adult mice and guinea pigs and loss of capacity to produce plaques or cytopathic effects in tissue culture. The suckling mouse was more sensitive for detecting live virus in irradiated suspensions than the adult mouse or guinea pig. Live virus was demonstrable in preparations exposed to 6 x 10^6 r but not in suspensions exposed to 8 x 10^6 r or more. The rate of inactivation of VEE virus by gamma radiation was an exponential function of the dosage.

14. Key Words
Venezuelan equine encephalitis
Radiation
Mice
Guinea pigs
Plaques
Tissue cultures
Suspension
Inactivation

DD FORM 1 JAN 64 1473

Unclassified
Security Classification