AD NUMBER

AD852034

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; APR 1969. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Release Branch/TIO, Frederick. MD 21701.

AUTHORITY

BDRL ltr, 29 Sep 1971

AD

TECHNICAL MANUSCRIPT 527

NONVIABLE VEE HEMAGGLUTININ PREPARED FROM TISSUE CULTURES BY GAMMA RADIATION

Morton Reitman

APRIL 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 527

NONVIABLE VEE HEMAGGLUTININ PREPARED FROM TISSUE CULTURES BY GAMMA RADIATION

Morton Reitman

Medical Investigation Division
MEDICAL SCIENCES LABORATORIES

Project 1B662706A072

April 1969

In conducting the research described in this report, the investigator 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.

ACKNOWLEDGMENTS

I thank James A. Hill, Leonard Green, and Antonio T. Marallo for valuable technical assistance.

ABSTRACT

Hemagglutinins (HA) of Venezuelan equine encephalomyelitis (VEE) virus were produced in Maitland-type cultures of chicken embryo (MTCE) and in monolayers of hamster kidney, McCoy, and human diploid strain Wl-38 cells. Optimal pH values for the demonstration of HA in MTCE preparations ranged from 5.65 to 5.85. Exposure of MTCE HA to 8×10^6 r of gamma rays destroyed the infectivity of the antigen while most of the HA activity was retained. Irradiated HA performed satisfactorily in hemagglutination-inhibition tests of human and animal sera.

NONVIABLE VEE HEMAGGLUTININ PREPARED FROM TISSUE CULTURES BY GAMMA RADIATION*

The hemagglutinin (HA) of Venezuelan equine encephalomyelitis (VEE) virus generally is prepared by cultivating the virus in the brain of the suckling mouse and then extracting the infected brain material with acetone and ether or sucrose and acetone to remove nonspecific inhibitors.[1] The demonstration of VEE HA in infected tissue cultures of chick embryo fibroblasts reported by Yershov and Vagzhanova[2] stimulated an investigation on the production of HA in tissue culture that would be applicable for routine titration of VEE hemagglutination-inhibiting (HI) antibodies.

The Trinidad donkey brain strain of VEE virus[3] was propagated in a Maitland-type chick embryo suspension (MTCE) prepared by suspending nine decapitated minced embryos in 300 ml of Nagle's defined medium[4]** containing 100 units of penicillin and 100 µg streptomycin per ml. The cultures, in 2-liter Fernbach flasks, were inoculated with 1 x $10^{6.3}$ mouse intracerebral 50% lethal doses ($MICLD_{50}$)/ml and were incubated on a reciprocating shaker for 18 hours. The cultures were filtered through sterile cotton gauze, clarified by centrifuging at 525 to 700 x g for 15 minutes, and stored in a mechanical freezer at -70 C. The virus suspension had a titer of 1 x $10^{9.9}$ $MICLD_{50}$/ml and $10^{9.3}$ mouse intraperitoneal 50% lethal doses ($MIPLD_{50}$)/ml.

Irradiation-inactivated viruses have been reported to retain most of their antigenicity,[5] and ionizing irradiation has been used to prepare noninfective complement-fixing antigens for influenza A, influenza B, mumps, smallpox, and herpes simplex, and HA antigen for influenza.[6] Polley[5] reported that gamma radiation was superior to formaldehyde treatment for preparing noninfective herpes simplex antigen. Irradiation of VEE virus suspensions with gamma rays has been shown to inactivate its infectivity.[7] Therefore, the use of ionizing radiation was investigated as an inactivating agent for the preparation of noninfective VEE HA.

Virus suspensions were exposed to radiation doses of 8 x 10^6, 10 x 10^6, and 16 x 10^6 r of Co^{60}, and were safety-tested for residual infectivity as described previously.[7]

Infectivity titers of 9.9 $MICLD_{50}$/ml were obtained in the VEE-infected MTCE cultures. HA titers of unirradiated virus suspensions determined by microtiter technique[1,8] ranged from 1:512 to 1:2048 per 0.05 ml. Exposure of virus suspensions to 8 x 10^6 r gamma rays destroyed the infectivity, but reduced the HA activity only fourfold (Table 1). Some HA activity was still present in the sample exposed to 16 x 10^6 r.

---

* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the author to ascertain when and where it may appear in citable form.

** USA-1 dehydrated, Grand Island Biological Co., Grand Island, N.Y.

TABLE 1. EFFECT OF IONIZING RADIATION ON VEE HEMAGGLUTININ

Lot No.	Titers[a] of HA Exposed to Roentgens x 10^6			
	0	8	10	16
MR 27	512	128	32	16

a. Reciprocal of dilution.

The optimal pH value for the HA test with MTCE-grown VEE ranged from 5.65 to 5.85. Courtney, Maney, and Smith[9] recently reported optimal pH values for HA of tissue culture grown arboviruses grown in tissue culture to be between 5.4 and 6.0.

Comparison of MTCE-irradiated antigen with a live sucrose-acetone-extracted suckling mouse brain antigen[1] and with a beta-propiolactone-treated suckling mouse brain antigen* in HI tests of human and animal sera did not reveal any significant difference in antibody titers.

Irradiated antigen (exposed to 8 x 10^6 r) has been employed routinely in our laboratory for the past 16 months in HI tests of animal and human sera with reproducible results. No change in HA titer was observed with antigen stored at -70 C or 4 C for 13 weeks or with antigen diluted one to ten in borate saline at -70 C for 13 weeks. Antigen stored at room temperature retained titer during an 8-day test period. Thawing and freezing eight times did not affect the HA titer, but an eightfold drop in titer was observed in one sample after storage at -70 C for 18 months.

The demonstration of arbovirus HA in tissue cultures has been reported for the virus of Japanese B encephalitis in hamster kidney cell monolayers;[10] for Semliki, Sindbis, and eastern equine encephalitis viruses in HeLa cell monolayers;[8,11] and for western equine encephalitis and yellow fever viruses in chick embryo fibroblasts and rabbit kidney cells.[9] I have investigated the production of VEE HA antigen in hamster kidney, McCoy, and human diploid cell strain WI-38 cell monolayers. High titers were obtained with hamster kidney (1:640) and McCoy (1:1,024) cell lines, but lower titers (1:40) were observed with WI-38 culture fluids.

The use of infected tissue culture fluids as a source of VEE HA antigen is recommended for routine laboratory HI tests. Irradiation with 8 x 10^6 r gamma rays appears to be an excellent means for preparing nonviable VEE HA. Several types of irradiation equipment have been marketed** that could be applicable to preparation of nonviable antigens. It is conceivable that in the near future many laboratories will have access to ionizing radiation sources.

* Allen, W.P., personal communication, 1967.

** Atomic Energy of Canada Limited Commercial Products, Ottawa, Canada.

LITERATURE CITED

1. Clarke, D.H.; Casals, J. 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Amer. J. Trop. Med. Hyg. 7:561-573.

2. Yershov, F.I.; Vagzhanova, V.A. 1965. Dynamics of multiplication of the VEE virus in tissue culture cells. Vop. Virusol. 2:176-180.

3. Hardy, F.M. 1959. The growth of Venezuelan equine encephalomyelitis virus in tissue cultures. Amer. J. Hyg. 70:21-27.

4. Nagle, S.C.; Tribble, H.R., Jr.; 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.

5. Polley, J.R. 1961. Preparation of non-infective soluble antigens with gamma radiation. Can. J. Microbiol. 7:135-139.

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

7. Reitman, M.; Tribble, H.R., Jr. 1967. Inactivation of Venezuelan equine encephalomyelitis virus by γ-radiation. Appl. Microbiol. 15:1456-1459.

8. Sever, J.L. 1962. Application of a microtechnique to viral serological investigations. J. Immunol. 88:320-329.

9. Courtney, R.J.; Maney, L.; Smith, J.S. 1967. Factors affecting the measurement of arbovirus hemagglutinin produced in tissue culture. Bacteriol. Proc. p. 167.

10. Hammon, W.McD.; Darwish, M.A. 1966. A high titered hemagglutinin in tissue culture prepared from Japanese B encephalitis virus. Proc. Soc. Exp. Biol. Med. 122:809-813.

11. Likar, M.; Buckley, S.M.; Clarke, D.H. 1962. Improved conditions for the production of arthropod-borne viral hemagglutinins in infected HeLa cell cultures. Virology 18:647-649.

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)	2a. REPORT SECURITY CLASSIFICATION
Department of the Army Fort Detrick, Frederick, Maryland, 21701	Unclassified 2b. GROUP

3. REPORT TITLE

NONVIABLE VEE HEMAGGLUTININ PREPARED FROM TISSUE CULTURES BY GAMMA RADIATION

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

5. AUTHOR(S) (First name, middle initial, last name)

Morton (NMI) Reitman

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
April 1969	9	11

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1B662706A072	Technical Manuscript 527
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10. DISTRIBUTION STATEMENT

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

Hemagglutinins (HA) of Venezuelan equine encephalomyelitis (VEE) virus were produced in Maitland-type cultures of chicken embryo (MTCE) and in monolayers of hamster kidney, McCoy, and human diploid strain WI-38 cells. Optimal pH values for the demonstration of HA in MTCE preparations ranged from 5.65 to 5.85. Exposure of MTCE HA to 8×10^6 r of gamma rays destroyed the infectivity of the antigen while most of the HA activity was retained. Irradiated HA performed satisfactorily in hemagglutination-inhibition tests of human and animal sera. ( )

14. Key Words

*Venezuelan equine encephalomyelitis virus
*Agglutinins
*Gamma rays
Radiation
Tissue culture
Chick embryo

DD FORM 1 NOV 65 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification