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EFFECTS OF GAMMA RADIATION ON WESTERN EQUINE ENCEPHALOMYELITIS AND TICK-BORNE ENCEPHALITIS VIRUSES

J. HRUŠKOVÁ

Military Institute of Hygiene, Epidemiology and Microbiology, Prague, Czechoslovakia

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Summary. — Gamma-irradiation caused complete loss of infectivity of Western equine encephalomyelitis (WEE) and tick-borne encephalitis (TE) viruses in medium 199 containing 2% calf serum, when applied in doses from $2\times10^6-3\times10^6$ rad. The rate of inactivation depended on the medium; it decreased somewhat with increased serum concentration. Lyophilized viruses were inactivated with a dose of 4×10^6 rad. Complete inactivation of the viruses by gamma-irradiation occurred before spontaneous virus inactivation had started. Samples irradiated with a dose of 4×10^6 rad showed decreased antigenicity.

Introduction

The effects of various types of ionizing radiation on a broad spectrum of viruses have been reviewed by McCrea (1960). Buthala (1962) tested the imunogenicity of beta-irradiated Herpesvirus simiae and found that it was unable to induce the formation of neutralizing antibody. The rabies vaccine obtained from mouse brains by electron irradiation (Friedemann, 1950) was highly immunogenic. Daneš and Blažek (1966) and Daneš *et al.* (1966) observed only a low antibody inducing capacity of Poxvirus officinale irradiated with a dose of 5×10^6 rad of gamma-rays. The antigenic properties of viruses are more resistant to irradiation than their infectivities (McCrea, 1960; Buzzell *et al.*, 1955).

In the present report we are summarizing the results of studies on the effects of gamma radiation on the infectivity and antigenicity of two arboviruses.

Materials and Methods

Viruses. WEE virus was obtained from Dr. Bárdoš, Institute of Epidemiology and Microbiology, Bratislava; it had undergone 10 chick embryo and 61 mouse brain passages. The Hypr strain of TE virus in its 38th mouse passage was kindly supplied by Dr. Kolman, Institute of Parasitology, Czechoslovak Academy of Sciences, Prague. The viruses were propagated in chick embryo cell (CEC) cultures; the culture fluids were harvested 24 and 48 hours after inoculation with WEE and TE virus, respectively.

Experimental animals. H strain mice wighing 10—13 g and guinea pigs weighing 250—300 g were used.

Cell cultures. CEC, obtained by trypsinization of 10-day chick embryos, were grown in medium 199 containing 3% calf serum (supplied by the Institute of Sera and Vaccines, Prague) and 200 units/ml penicillin plus 200 μg/ml streptomycin.

Irradiation was carried out in a Gammacell 220 apparatus (Atomic Energy of Canada, C.P.Ltd., Ottawa) at room temperature and a flux of 4×10^3 rad/min. The virus samples were irradiated in 50- or 100-ml polyvinyl chloride bottles. The doses of radiation are expressed in rad units.

Virus titration. WEE virus was titrated in CEC cultures either in tubes according to the cytopathic effect read after 3—4 days or in Petri dishes by the plaque method (Vonka, 1965). The results were expressed in TCD_{50} or PFU per 0.1 ml values, respectively. TE virus was titrated either by the plaque method in CEC or by intracerebral inoculation of mice with 0.03 ml volumes (LD_{50} values were calculated according to the formula of Reed and Muench).

Serological reactions. The haemagglutination inhibition (HI) tests were carried out according to Clarke and Casals (1958). Neutralizing antibody against WEE virus was assayed by the plaque method; the antibody titre was expressed by the highest dilution of serum that caused 80% WEE virus plaque inhibition. Neutralizing antibody against TE virus was assayed in mice intraperitoneally and its level expressed by the neutralization index of serum. The complement-fixation (CF) test was carried out in the cold with antigen prepared from mouse brains by ether extraction (Ilyenko, 1953).

Protection test. Immunized mice were challenged with 10-fold serial dilutions of virus, administered in 0.2 ml volumes intraperitoneally (WEE) or 0.1 ml volumes subcutaneously (TE). The results were expressed in the form of protection indices.

Results

Inactivation of WEE and TE viruses

WEE virus (Fig. 1). Four experiments were carried out to establish the rate of virus inactivation in medium 199 containing 2% calf serum and to determine the dose causing safe inactivation. The virus titre started to decrease after a dose of 10^5 rad and after about 2×10^6 rad the virus was completely inactivated. Only exceptionally were traces of virus found after irradiation with up to 2.6×10^6 rad, but after irradiation with 3×10^6 rad not even traces of live virus were ever demonstrated.

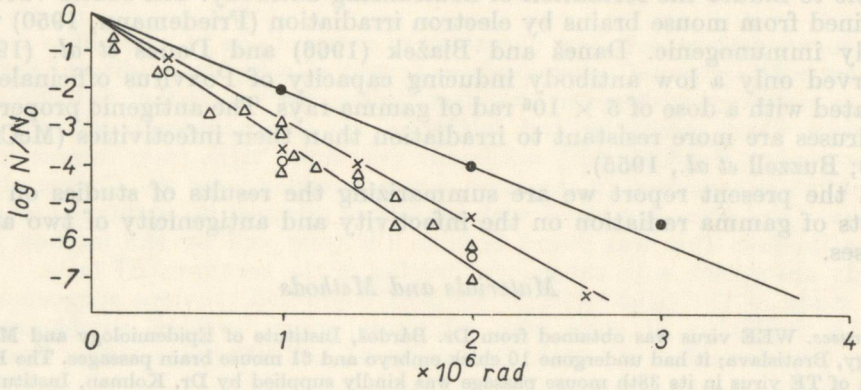

Fig. 1.

Inactivation of WEE virus in different media by gamma radiation

Abscissa: dose of radiation; ordinate: log N/N_0 (N and N_0 = amount of virus before and after irradiation, respectively)

○ Medium 199 + 2% calf serum
× Medium 199 + 40% calf serum
● Lyophilized virus
△ Summarized results of 4 experiments in medium 199 + 2% calf serum

In another experiment we compared the inactivation rates of WEE virus in different media (medium 199 with either 2% or 40% calf serum) and of virus lyophilized with 50% calf serum. Inactivation was the most rapid in medium containing 2% calf serum, the results being in accordance with those mentioned above. Inactivation of lyophilized virus proceeded more slowly, but the difference in the endpoints was only about 10^6 rad.

TE virus (Fig. 2). Four experiments on virus inactivation in medium 199 with 2% calf serum were carried out. The virus titrated by the plaque method (2 experiments) or in mice (2 experiments). The virus titres started to decrease on irradiation with 10^5 rad and complete inactivation occurred with about 2×10^6 rad. At a dose of 2.5×10^6 rad, not even traces of live virus were ever demonstrated.

Complete inactivation of WEE and TE viruses by gamma rays was achieved in a sufficiently short time, before spontaneous virus inactivation could have taken place.

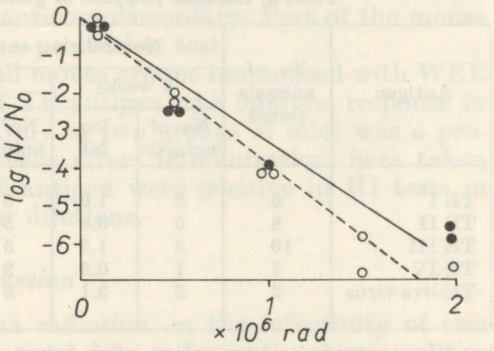

Fig. 2.

Inactivation of TE virus in medium 199 with 2% calf serum by gamma radiation
Abscissa and ordinate as in Fig. 1.
●———● Surviving virus titrated by the plaque method in CEC cultures
○-------○ Surviving virus titrated in mice

Testing of antigenicity of the inactivated viruses

Viruses in medium 199 with 2% calf serum, inactivated with a dose of 4×10^6 rad, were used for immunization. Inactivation was checked with WEE virus by the plaque method in CEC cultures and with TE virus both by the plaque method and in mice simultaneously inoculated with 0.2 ml intraperitoneally and 0.03 ml intracerebrally. In the plaque method, each dish culture was inoculated with 4 ml. In this way, one quarter of each sample used for immunization was tested. Live virus was never demonstrated.

Four samples of either inactivated antigen were examined. The virus titres before irradiation

Table 1. Immune response of guinea pigs to irradiated WEE antigen

Antigen	No. of animals tested	Neutralizing antibody				HI antibody				CF antibody			
		2 weeks*		4 weeks		2 weeks		4 weeks		2 weeks		4 weeks	
		I	II	I	II	I	II	I	II	I	II	I	II
WEE I	6	1	5	1	10	2	80	1	20	2	4	1	4
WEE II	6	4	5	4	10	6	320	6	320	4	64	4	32
WEE III	6	2	10	2	20	5	320	6	160	5	32	4	8
WEE-live virus	1	1	320	1	640	1	1280	1	2560	1	256	1	128

* Weeks after immunization.
I = No. of animals positive for antibody (with titres of at least 2, 10 and 4 in the neutralization, HI and CF tests, respectively).
II = Maximal antibody titres (serum dilution reciprocals).

were 8.5, 8.7, 7.7 and 8.3 log TCD_{50}/0.1 ml in samples WEE I, II, III and IV, respectively, and 6.7, 5, 6 and 5.7 log ic mouse LD_{50}/0.03 ml in samples TE I, II, III and IV, respectively.

Guinea pigs were immunized with 3 doses of 1 ml antigen at weekly intervals, either subcutaneously or intraperitoneally. Blood samples for serological examination were taken before immunization and 2 and 4 weeks after the 3rd dose.

Table 2. Immune response of guinea pigs to irradiated TE antigen

Antigen	No. of animals tested	Neutralizing antibody				CF antibody			
		2 weeks		4 weeks		2 weeks		4 weeks	
		No. of animals	log NI	No. of animals	log NI	No. of animals	Max. titre	No. of animals	Max. titre
TE I	6	5	1.0	5	1.1	2	4	2	8
TE II	8	5	0.8	5	1.2	2	4	0	
TE III	10	5	1.5	5	2.0	1	8	1	4
TE IV	7	1	0.5	3	1.0	0		0	
TE-live virus	2	2	3.1	2	4.5	2	128	2	128

log NI = maximal neutralization index values. Values < 0.5 were considered negative.
For other explanations see Table 1.

The results of serological investigations showed that the ability of inactivated virus antigens to induce antibody formation in guinea pigs was partially lowered with WEE virus (Table 1) and almost destroyed with TE virus (Table 2). HI antibodies were never found in guinea pigs immunized

Table 3. Immune response of mice to irradiated WEE and TE antigens

Antigen	Mouse group	Neutralizing antibody**		HI antibody		Protection index	
		2 weeks	3 weeks	2 weeks	3 weeks	2 weeks	3 weeks
WEE I	A	2	—	80	—	2.8	—
	B	< 2	—	40	—	1.8	—
WEE II	A	5	—	80	—	3.6	—
	B	5	10	20	20	1.9	2.2
WEE III	A	80	—	80	—	—	—
	B	10	—	20	—	—	—
	C	—	2	—	80	—	—
WEE IV	A	< 0.5	5	< 10	< 10	1.0	4.6
TE I	A	0.5	1.0	20	10	1.7	2.0
TE II	A	< 0.5	< 0.5	< 10	10	< 0.5	0.7
TE III	A	1.2	< 0.5	< 10	20	1.0	2.0
TE IV	A	0.7	0.5	< 10	20	< 0.5	< 0.5

* A — Mice immunized with 3 intraperitoneal doses of 0.2 ml each at weekly intervals.
B — Mice immunized with 3 subcutaneous doses of 0.5 ml each at weekly intervals.
C — Mice immunized with 1 intraperitoneal dose of 0.5 ml.
** WEE: serum dilution reciprocals; TE = log NI of serum.
— = Not done.

with inactivated TE virus antigen and these negative results were omitted from Table 2.

Mice in groups of 80 animals each were immunized with inactivated antigens as indicated in Table 3. Two and three weeks after the end of immunization, 15 mice of each group were exsanguinated. The sera were examined in virus neutralization and HI tests. The CF reaction was not evaluable, because most of the mouse sera were anticomplementary. Part of the mouse groups was tested for resistance in the protection test.

An immune response was found in all mouse groups immunized with WEE antigens. Following administration of TE antigen, the immune response in mice was slight, like in guinea pigs. Only in two groups of mice was a protection index of 2 log found three weeks after immunization. Sera taken 3 weeks after immunization with TE antigen were positive in HI tests in all four mouse groups, but only in low dilutions.

Discussion

Our results on the effect of gamma radiation on the infectivity of two arboviruses are in accordance with reported data as far as the characteristics of the course of inactivation are concerned. Moreover, they are in accordance with results of those authors who found that higher doses of ionizing radiation are necessary for complete inactivation of lyophilized viruses as compared with liquid samples. Huber (1952) found no difference between the radiosensitivity of liquid, frozen and lyophilized virus materials; his results suggest that a higher resistance of dry materials needs not be a general rule. It is possible that Huber worked with a virus considerably resistant to indirect side effects of irradiation so that the degree of hydratation did not manifest itself.

In the second group of experiments we found a certain degree of immunogenicity of the irradiated arboviruses, a higher one in WEE antigen and a little marked one in TE antigen. Our results cannot be compared with others, because so far no data have been reported on the immunogenicity of arboviruses inactivated by ionizing radiation. The lower activity of TE virus antigens than that of WEE virus antigen probably cannot be unequivocally explained by a lower virus content in the starting suspensions before irradiation. Since the two viruses were titrated in different systems, it appears impossible to estimate the difference in the amount of virus in the respective preparations. Differences in virus titres of the individual WEE virus samples did not affect their immunogenicity, however.

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