Inactivation of Some Animal Viruses with Gamma Radiation from Cobalt-60.* (22215)

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The need for obtaining better methods of inactivating viruses for preparing non-infectious vaccines and other biologicals indicates the desirability of exploring more efficient means of virus inactivation. While some information has been accumulated concerning the effect of small doses of alpha, beta and gamma radiation on viruses(1), little data that deal with sterilizing doses are available. Huber(2) has reported that many virus preparations can be inactivated by 40000 to 1400000 roentgen equivalent physicals (rep), using high intensity electron beams from a capacitron. Nickerson(3) has indicated considerably higher sterilization doses for viruses, varying from 1 to 20 million rep.

Studies described here deal with the effect of gamma radiation on 4 animal viruses *in vitro*. The experiments were designed to determine the dosage necessary to render these viruses non-infectious when irradiated in whole brain and in suspension. Since it has been suggested(1) that a relationship may exist between size of viruses and their resistance to ionizing radiations, the viruses used were selected to cover a range of particle size.

Materials and methods. At the time this study was initiated, poliovirus was selected as representative of a small virus. St. Louis encephalitis (SLE) and Western equine encephalomyelitis (WEE) viruses were chosen to represent medium sized viruses, and vaccinia a large virus. More recent data, however, indicate that poliovirus is somewhat larger than was previously believed and approximates the size of St. Louis virus(4). *Viruses* used and their approximate diameters were as follows: California strain of WEE virus 53 mμ(5), Hubbard strain of SLE virus 20-30 mμ(6) obtained from American Type Culture Collection. Armstrong 1166 neurotropic strain of vaccinia virus 225 mμ(7), Lansing strain of poliomyelitis virus 22 to 27 mμ diameter(4) were kindly supplied by the University of Michigan School of Public Health. All *virus suspensions* were prepared from infected brains of 3- to 4-week-old Swiss mice. Ten percent brain suspensions were prepared by grinding with alundum and cold physiological saline. Suspensions were subjected to the following cycles of treatment: (a) sedimentation at 2000 rpm for 5 minutes; the supernatant fluid was used as crude virus (CV), (b) sedimentation of CV at 3000 rpm for 10 minutes, (c) sedimentation of second supernatant fluid at 4000 rpm for 15 minutes, (d) filtration of third supernatant fluid through a Seitz EK pad, (e) sedimentation of the filtrate at 45000 rpm for 1.5 hours and reconstitution of the pellet in pH 7.2, 0.05 M phosphate buffer, (f) sedimentation of the reconstituted material at 4000 rpm for 15 minutes. Alternate high and low speed centrifugations were repeated until the virus preparation had been washed 3 times. Immediately after the final wash the virus pellet was resuspended in 2 ml of phosphate buffer to make a concentrated suspension of partially purified virus (PPV). One ml aliquots of all virus suspensions were placed in glass plastic-capped vials and shell frozen in a dry-ice-alcohol bath. Vials containing the frozen virus were then stored at −50°C until they were irradiated. Samples of whole mouse brains were frozen and stored in the same manner. *Titration of viruses.* The various virus suspensions were titrated for infectivity by intracerebral injection of 3- to 4-week-old mice using .05 M phosphate buffer as diluent. The LD_{50} titers were determined by the method of Reed and Muench(8). The various virus

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TABLE I. *Rates of Inactivation of Poliovirus, St. Louis Encephalitis, Western Equine Encephalomyelitis and Vaccinia Viruses in Whole Brain, Crude Suspensions and Partially Purified States.*

Virus	Diameter, mμ	Virus sample	Unirradiated controls	rep* radiation in millions							
				1	1.5	2	2.5	3	3.5	4	4.5
Poliovirus	22–27	WB†	4.3‡	3.3	3.0	2.3	1.9	1.3	0	0	0
		CV	6.0	4.6	4.0	3.3	2.6	2.2	1.4	0	0
		PPV	6.0	4.2	3.3	2.5	1.7	0	0	0	0
SLE	20–30	WB	7.2	5.8	5.1	4.3	3.5	3.1	2.2	1.5	0
		CV	6.0	4.6	4.0	3.3	2.7	2.1	1.4	0	0
		PPV	6.0	4.2	3.3	2.5	1.6	0	0	0	0
WEE	53	WB	8.1	6.3	5.4	4.8	4.0	3.2	2.3	1.6	0
		CV	6.0	4.4	3.6	3.1	2.2	1.6	0	0	0
		PPV	6.0	4.0	2.8	2.0	0	0	0	0	0
Vaccinia	225	WB	5.2	3.7	2.6	1.8	0	0	0	0	0
		CV	6.0	4.1	3.0	1.9	0	0	0	0	0
		PPV	6.0	2.5	0	0	0	0	0	0	0

* Roentgen equivalent physicals. † WB—whole brain, CV—crude virus, PPV—partially purified virus. ‡ All figures represent log of LD_{50} dilution.

suspensions were diluted to desired LD_{50}, just before they were irradiated. *Method of Irradiation.* Samples were removed from the deep freeze and placed in the center well of the large cobalt-60 source housed in the Fission Products Laboratory of the University of Michigan. Exposure rate of gamma rays emitted was approximately 200000 roentgen equivalent physicals (rep) per hour(9). One rep as defined for tissue in air represents an energy absorption dose of 93 ergs/g. Ferrous-ferric dosimetry was used for calibration. The calibration solution was placed in vials similar to those used for virus preparations and readings were based on oxidation of 15.4 μ moles of ferrous ions/liter/1000 rep. Uniform doses were attained by rotating samples on a horizontal plane in the center well of the Cobalt source. Samples to be tested were irradiated from 5 to 24 hours, during which time they were kept frozen with dry ice at approximately −72°C. All virus samples to be irradiated for a given experiment were placed in the center well at the same time. After the required exposure, samples were removed and immediately tested for presence of virus by intracerebral titration in mice, using 10 mice for each of an appropriate series of 10-fold dilutions. Control samples of virus were exposed to identical conditions but were not irradiated.

Results. As a first step toward studying the effect of gamma rays on animal viruses, samples of virus preparations were exposed to cobalt-60 radiations from 1 to 4.5 million rep. Frozen CV and PPV suspensions diluted (to the same volume) in 0.05 M phosphate buffer to contain the same number of LD_{50}, and whole brain preparations were irradiated under conditions described. During irradiation, samples of virus were removed at intervals computed to yield doses of gamma rays as indicated in Table I. Each sample was tested for reduction in virus titer by intracerebral injection in mice.

It may be seen that WB preparations for all viruses tested required longer periods of irradiation than did either CV or PPV suspensions. Crude suspensions of all viruses were inactivated at a significantly faster rate than were PPV suspensions. Results obtained with SLE virus are almost identical to those obtained with poliomyelitis virus. Comparison of these data show that both SLE and poliovirus required 3.5 to 4 million rep before CV suspensions were rendered non-infectious, and that PPV samples lost their infectivity at 2.5 to 3 million rep. From data presented it is evident that crude suspensions of WEE virus required less irradiation before infectivity was lost than did comparable suspensions of SLE or poliovirus. Similarly, PPV suspensions of Western virus were reduced in infectivity at a faster rate than were the SLE and Lansing viruses. Comparison of data on PPV suspensions reveals that West-

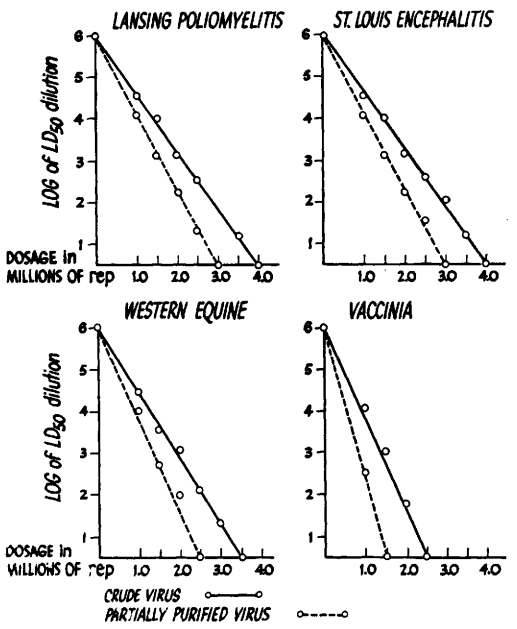

FIG. 1. Survival curves of gamma irradiated viruses in crude and partially purified suspensions.

ern PPV was no longer infectious after 2.5 million rep of irradiation. Partially purified SLE and poliovirus, on the other hand, required an additional 500000 rep before suspensions lost their pathogenicity for mice. It appears that inactivation of vaccinia virus by gamma radiation followed the same general sequence as that described for SLE, WEE, and poliovirus.

To show graphically the rates of inactivation for CV and PPV suspensions containing the same number of LD_{50}, the data in Table I were plotted on semi-logarithmic paper and presented in Fig. 1. The points on these curves are averages of virus dilution expressed as logarithms of the LD_{50}. The slopes of the curves suggest that purification of viruses removed some substance which protected the virus particle from the lethal effects of gamma rays. When the surviving virus fractions, or LD_{50}, are plotted on a logarithmic scale, the points are found to lie approximately on a straight line showing that, within error of the experiment, the surviving fraction is an exponential function of the dose. It should be pointed out that although error of assessment of virus activity may sometimes be rather large, there appear to be no constant deviations from exponential survival as distinct from random variations.

Discussion. The relationship between virus size and dose of gamma radiation required for inactivation is seen in Table I. The data show that CV suspensions of poliomyelitis and SLE virus required the same dosage of radiation before they were inactivated, while vaccinia virus was rendered non-infectious by a significantly smaller dose. Inspection of the data obtained with PPV suspensions suggest that smaller virus particles require larger doses of radiation for inactivation than do larger virus particles. It appears that the differential in size between poliovirus and SLE is not sufficient to make a difference in the dose of cobalt radiation needed for inactivation.

Of the PPV suspensions tested it may be seen that SLE and poliovirus with diameters of 20-30 mμ required 3 million rep for complete inactivation while the intermediate WEE virus required a slightly smaller dose. Vaccinia, the largest virus used, was inactivated with half the radiation required for the smallest viruses.

Preliminary experiments indicate that 2 to 3 times the dose of cobalt-60 required for inactivation does not impair the antigenicity of any of the viruses tested, and that gamma irradiated antigens elicit an immunologic response comparable to that induced by chemically inactivated viruses. These experiments will be presented in detail in a subsequent report.

Summary. 1. Gamma radiation from cobalt-60 proves to be an effective method for inactivation of Lansing poliomyelitis, St. Louis encephalitis, Western equine encephalomyelitis and vaccinia viruses. 2. Partially purified suspensions of viruses tested were more vulnerable to lethal effects of gamma radiation than were crude suspensions. 3. In crude and partially purified suspensions the smaller viruses required larger doses of gamma radiation for inactivation than did the larger viruses. 4. The rate of inactivation for viruses tested, within error of experiment,

is an exponential function of the dosage of gamma radiation.

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Infection of *Mansonia perturbans* and *Psorophora ferox* Mosquitoes with Venezuelan Equine Encephalomyelitis Virus. (22216)

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Past studies have indicated Venezuelan equine encephalomyelitis (VEE) to be a mosquito-borne disease. In Trinidad, Gilyard(1) transmitted VEE from donkey to donkey by bite of *Mansonia titillans* (Walker). In Ecuador, Levi-Castillo(2) reported transmission by *Aedes taeniorhynchus* (Wied.), *M. titillans,* and *Culex quinquefasciatus* (Say). Recent work in the United States(3-5) has shown *A. triseriatus* (Say) to transmit efficiently in the laboratory.

The present report concerns studies on the VEE vector potentials of two additional species of mosquitoes, *M. perturbans* (Walker) and *Psorophora ferox* (Humboldt).

Materials and methods. The strain of VEE virus used, and methods of virus titration and of handling, feeding, incubating, and processing the mosquitoes were the same as reported previously(3,4). The *M. perturbans* were collected alive in Louisiana as adults, and were used in experiments 5 days later. The *P. ferox* were reared from larvae collected near Montgomery, Ala., and were 7-10 days old when used. Adult guinea pigs inoculated intraperitoneally with 1,000-10,000 mouse LD_{50} of virus 48 hours previously served as sources for mosquito infection. After incubation at 80°F, the mosquitoes were refed individually upon normal 3-week-old guinea pigs to determine their ability to transmit infection by bite.

Results. M. perturbans. Forty females engorged upon blood having a mouse intraperitoneal virus titer of $10^{8.4}$. A total of 16 egg rafts were laid by these specimens 6-13 days later. These rafts were ground in 2 ml of diluent each and titrated for virus. On the 13th day of incubation 16 of 29 mosquitoes remaining alive refed individually upon normal guinea pigs. These mosquitoes, as well as those which did not refeed, were then titrated to determine the amount of virus each contained. Table I summarizes the infection and transmission data.

P. ferox. Fifty females were fed upon blood containing virus titering $10^{6.3}$. Several hundred eggs laid 6-11 days later were pooled, ground in 2 ml of diluent, and inoculated into mice to test for virus.

On the 14th day of incubation the surviving mosquitoes were given opportunity to refeed individually upon normal guinea pigs. Subsequently, all mosquitoes were titrated to determine the amount of virus contained per