

# Gamma Radiation Inactivation of Coxsackievirus B-2

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The radioresistance of coxsackievirus B-2 was studied when the virus was suspended in Eagle minimal essential medium, distilled water, cooked ground beef, and raw ground beef and irradiated at various temperatures in a cobalt-60 gamma radiation source. The number of surviving viruses at given doses of radiation was determined by a plaque assay system. All destruction curves indicated a first-order reaction. When the virus was irradiated in minimal essential medium at temperatures of -30, -60, and -90 C, *D* values (in Mrad) were 0.69, 0.59, and 0.64, respectively. When the virus was suspended in water and irradiated at -90 C, the *D* value was 0.53. Cooked ground beef containing the virus was irradiated at temperatures ranging from 16 to -90 C. The *D* values were 0.70 (16 C), 0.76 (0.5 C), 0.68 (-30 C), 0.78 (-60 C), and 0.81 (-90 C). Raw ground beef containing the virus was irradiated at -30, -60, and -90 C, and the *D* values were respectively 0.75, 0.71, and 0.68. The *D* values indicate that the rate of viral inactivation was dependent on the suspending medium.

The use of gamma radiation has been advocated as a means of obtaining sterile, organoleptically acceptable raw and cooked foods that require no refrigeration storage and have a long shelf life (10). Safety, enzymatic changes, and microbiological considerations are factors that affect any such food processing system. Present systems utilize low temperature heating followed by irradiation of the frozen (-20 to -40 C) food product.

A number of viruses capable of infecting man have been isolated from food and food animals (1, 2, 4, 5, 9, 11, 12, 16, 20, 22, 23, 29, 31), and extensive studies on viral inactivation by gamma radiation have been reported (3, 6, 7, 15, 18, 19, 24, 25, 26-28). However, only one investigation dealt with inactivation of viruses in food processing. A 99% reduction of poliovirus in fish fillets was observed after an irradiation dose of 0.6 Mrads (14).

The limited information on inactivation of viruses in foods prompted a study to determine the safety of such foods, and to obtain base line data on viral inactivation. Coxsackievirus B-2 was suspended in Eagle minimal essential medium (MEM) in Hanks balanced salt solution (8, 13), distilled water, and ground beef and

irradiated at temperatures ranging from 16 to -90 C. The results indicate that the rate of viral inactivation is dependent on the suspending medium.

## MATERIALS AND METHODS

**Virus.** Coxsackievirus B-2, Ohio 1, VR-29 was passaged in continuous cell cultures (Vero) from African green monkey kidney (*Cercopithecus aethiops*; reference 32) by using L-15 medium (17) supplemented with 2% fetal bovine serum and 0.07% NaHCO<sub>3</sub>. Cell monolayers showing advanced cytopathic effects were frozen and thawed three times, and the virus was harvested. The harvest was clarified by centrifugation for 15 min at 1,060 × *g* at 4 C. The virus titer was determined, and the harvest was dispensed into borosilicate glass ampules; the ampules were flame-sealed and stored at -60 C. This procedure provided a virus pool of known titer for use throughout the investigation.

**Virus assay.** A plaque forming unit (PFU) assay system was used as previously described (30). This system consisted of an agar-medium overlay and monolayer Vero cell sheets (45 cm<sup>2</sup>) in 6-oz (approximately 0.17 liter) bottles.

**Radiation source.** A 2,800-C, cobalt-60 gamma radiation source was used. The irradiation chamber consisted of 36 cobalt-60 containing pins immersed in 4 meters of deionized water. An overhead device was

used to position and rotate a cylinder that housed a rig containing the samples of virus materials. Temperatures were maintained during radiations by metering gaseous nitrogen from a liquid nitrogen tank into the irradiation rig.

Vertical positioning on the rig of the tubes containing the material being irradiated permitted delivery of eight different doses in a given time. These doses, in Mrad, were 0.43, 0.81, 1.35, 1.88, 2.04, 2.21, 2.67, and 2.82. The Mrad at each tube position in the radiation rig were determined by cobalt glass dosimetry. During dosimetries, cobalt glass strips were placed in tubes containing MEM plus 2% fetal bovine serum, distilled water, cooked ground beef, and raw ground beef. No significant differences were seen in the delivered doses among the menstra.

**MEM and water irradiation samples.** The virus was diluted to approximately 10,000 PFU/ml in MEM (pH 7.0) containing 2% fetal bovine serum, or in distilled water. Next, 1.2 ml of the virus-containing liquid was pipetted into 13 by 53-mm borosilicate glass tubes, which were then flame-sealed. Tubes containing the virus material were equilibrated at the temperature at which they were to be irradiated. Temperatures during irradiation runs were -30, -60, or -90 C. A 1-ml sample from each tube was assayed for viral PFU.

**Ground beef.** The ground beef used in this study was obtained from one animal and was processed, ground, and canned under clean-room conditions at Natick Laboratories, Natick, Mass. The beef was U.S. grade choice, boneless chuck that was ground through a  $\frac{3}{16}$ -in. (approximately 0.46 cm) plate twice; the fat content was 25%. Twenty-six cans containing 400 g each of meat were vacuum sealed and frozen at -30 C. A similar number of cans were prepared with the same batch of meat, sealed, heated to 80 C, and held at this temperature for 15 min. These cans were then stored at -30 C. The unheated meat was designated as raw ground beef, and the heated meat was designated as cooked ground beef. All cans of meat were held at -30 C until used.

**Meat irradiation samples.** One-gram portions of the meat containing approximately 10,000 PFU of coxsackievirus B-2 were placed in individual 13 by 53-mm borosilicate glass tubes, which were then flame-sealed (29). The sealed tubes were held at -60 C until used in an irradiation run. Representative samples were assayed for viral PFU to ensure even distribution of coxsackievirus B-2 among the 1-g portions of the meat prior to irradiation.

Meat-virus samples held at a given temperature during irradiation were allowed to equilibrate at this temperature before being lowered into the cobalt-60 well. Four samples were used at each dose in each irradiation run.

When an irradiation run was completed, the samples were stored at -60 C until assayed along with positive and negative controls of the same run. All control samples received treatment identical to that received by the irradiated samples, except that the control samples were not irradiated.

**Viral assay of meat.** A method previously shown to give satisfactory virus recovery from ground beef was used (29). This method consisted of making meat slurries by shaking 1-g portions of ground beef in MEM (pH 8.5) and clarifying these slurries by passing the liquid portion through cheese cloth.

The filtrate from each 1-g sample was individually titrated by  $\log_{10}$  dilution steps when necessitated by the anticipated PFU number. One-milliliter portions from each dilution were assayed in each of four 6-oz (approximately 0.17 liter) bottles containing Vero cell monolayers. This quadruplicate assay was done for 1-g portions of meat irradiated at lower dose levels; i.e., 1.35 Mrad and lower. At doses of 1.88 Mrad and higher, all of the meat filtrate was assayed. This was done by assaying equal portions of the filtrate in each of four 6-oz (approximately 0.17 liter) prescription bottles containing monolayers of the monkey kidney cells.

**Calculation of D values.** The D value is the dose of gamma radiation that reduces the viral population by 90%. D values were calculated by the following formula. A linear model was assumed, and the parameters  $\beta_0$  and  $\beta_1$  were estimated for each run. The model was:  $Y = \beta_0 + \beta_1 X + \epsilon$ , where  $Y = \log_{10}$  plaque count,  $\beta_0$  and  $\beta_1 =$  true but unknown regression coefficients,  $X =$  gamma radiation in Mrad, and  $\epsilon =$  experimental error. This model was used to obtain an estimate for viral radioresistance, and goodness-of-fit tests were performed to confirm the choice of model. The value of one over the estimate of the slope ( $\beta_1$ ) and its confidence intervals are the D value in Mrad and its confidence intervals.

## RESULTS

A series of experiments was done to determine the effect of suspending medium and temperature on coxsackievirus B-2 during irradiation. The radioresistance data for coxsackievirus B-2 in MEM plus 2% fetal bovine serum, distilled water, and ground beef are presented in Table 1.

All data were linear over the range studied in that more than 90% of the sum of squares was explained by the model. An example of plotted data is presented in Fig. 1. There was little change with temperature among D values of coxsackievirus B-2 irradiated in frozen MEM with 2% fetal bovine serum or in ground beef.

The data from the irradiation runs on the virus in cooked and raw ground beef were analyzed to determine if the slopes of the regression lines were equal (21). None of the variance-ratio values, computed to test the hypothesis that the slopes among runs were equal, exceeded the critical value at  $\alpha = 0.01$ . These analyses indicated that there were practically no differences among the slopes of the curves for irradiation runs at the given temperatures investigated.

TABLE 1. *Gamma radioresistance of coxsackievirus B-2 in Eagle minimal essential medium, distilled water, and ground beef*

Virus-containing material	Irradiation temp (C)	D value (Mrad)	99% Confidence limits	Observations (no.)	Runs (no.)
MEM <sup>a</sup> plus 2% fetal bovine serum	0.5 <sup>b</sup>	0.45	0.42-0.48	106	4
	-30	0.69	0.54-0.94	8	1
	-60	0.59	0.35-1.93	6	1
	-90	0.64	0.49-0.91	9	1
Distilled water	0.5 <sup>b</sup>	0.14	0.10-0.21	11	1
	-90	0.53	0.47-0.62	26	1
Cooked ground beef	16	0.70	0.66-0.74	170	6
	0.5	0.76	0.74-0.79	187	6
	-30	0.68	0.63-0.72	175	6
	-60	0.78	0.72-0.84	165	6
	-90	0.81	0.77-0.85	185	6
Raw ground beef	-30	0.75	0.70-0.81	49	2
	-60	0.71	0.64-0.80	64	2
	-90	0.68	0.62-0.77	38	2

<sup>a</sup> MEM, minimal essential medium.

<sup>b</sup> These irradiation runs at 0.5 C were previously reported (28). The data are included here for comparison purposes.

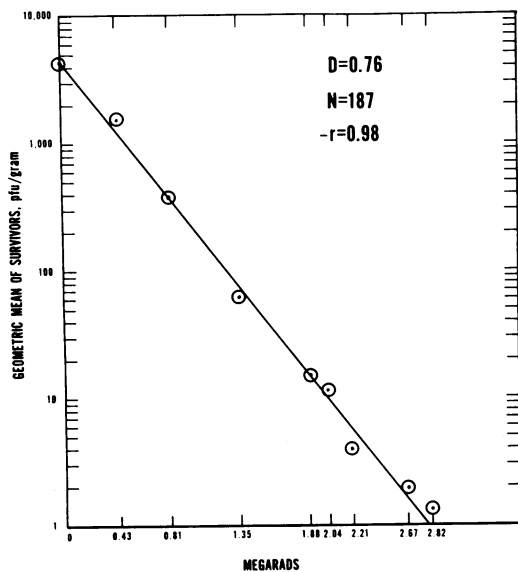


FIG. 1. *Gamma radiation inactivation of coxsackievirus B-2 in cooked ground beef at 0.5 C. D is the dose in megarads that reduced the viral plaque forming units (PFU) by 1 log<sub>10</sub>. N is the number of 1-g meat-virus samples assayed, and r is the correlation coefficient.*

## DISCUSSION

Coxsackievirus B-2 was suspended in MEM-serum medium, distilled water, cooked ground beef, and raw ground beef and irradiated at

different temperatures to determine the effect of suspending medium and temperature on viral inactivation.

The limited number of observations in the MEM irradiation runs precluded tight 99% confidence limits. However, computed D values indicated no large difference in the rate of viral inactivation among irradiation runs at -30, -60, and -90 C. D values at the three temperatures were higher than the previously reported D value (0.45 Mrad) computed for the same virus in the same medium at 0.5 C (28). When the virus was suspended in water and irradiated at -90 C, the D value of 0.53 Mrad was significantly greater than the previously reported D value of 0.14 Mrad when the virus was irradiated in water at 0.5 C (28). The higher D values observed for the virus in the frozen material could be due to the inhibition of free-radical formation or to impeding of free-radical travel in the frozen material. The presence of free-radical scavengers, such as fetal bovine serum in the Eagle MEM appears to be related to the higher D value (0.45 Mrad) observed when the virus was irradiated at 0.5 C in this medium as compared to the D value (0.14 Mrad) of the virus irradiated at the same temperature in distilled water (28).

No trend in D values with temperature was seen when the virus was suspended in raw ground beef and irradiated at temperatures ranging from -30 to -90 C, nor was any trend in D values with temperature noted in cooked

ground beef irradiated at temperatures ranging from 16 to  $-90^{\circ}\text{C}$ . Apparently, there is enough free radical scavenging by proteins and other substances in the ground beef to eliminate or reduce the secondary effects of radiation. This is clearly illustrated when the D value of 0.76 Mrad in cooked meat irradiated at  $0.5^{\circ}\text{C}$  is compared with the D value of 0.14 Mrad in water irradiated at the same temperature.

If the 12-D concept is used to calculate a food process, the dose required for gamma-radiation sterilization is 12 times the D value. As an example: cooked ground beef containing coxsackievirus B-2 irradiated at  $-30^{\circ}\text{C}$  requires 12 times 0.68 or 8.16 Mrad. The D values reported apply only to the menstra, temperatures, and virus investigated. There are variations in the composition of foods and other viral suspending media; however, the reported D values for coxsackievirus B-2 could be utilized as a starting point for viral inactivation studies.

#### ACKNOWLEDGMENT

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