Effect of Temperature on Radiosensitivity of Newcastle Disease Virus

G. A. DiGIOIA, J. J. LICCIARDELLO, J. T. R. NICKERSON, AND S. A. GOLDBLITH
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received for publication 3 November 1969

Newcastle disease virus was irradiated at temperatures ranging from 2.2 to 60 C. An interaction between the thermal and ionizing energy was observed in the temperature region of 49 to 60 C. At 2.2 C, the hemagglutinin was considerably more radio-resistant than the infectivity property. It is believed that radiation inactivation of Newcastle disease virus infectivity at low temperatures was due to nucleic acid degradation and at higher temperatures was due to protein denaturation.

The effect of ionizing radiation on viral inactivation has been studied since as early as 1939 (5). The subject matter has been discussed in great detail by Lea (8) in his classic book, Action of Radiations on Living Cells, in which the target theory was developed. Research in this area has continued, but the main emphasis of this research has been on the use of ionizing radiation as a tool to study molecular organization of viruses, particularly in relation to biological function, or particle size, or for preparing vaccines. Little consideration has been given to radiation as a means of decontaminating virus-infected foods because of the relatively high sterilizing dose required (7) which results in undesirable organoleptic changes. Thus, any treatment that might sensitize virus to radiation warrants an investigation.

It was reported that the survivors of X-irradiated T5 bacteriophage are more sensitive to heat (2). Adams and Pollard (1) similarly found that irradiation of T1 bacteriophage particles sensitized them to heat, but of more importance was the finding that a greater degree of inactivation occurred when the irradiation and heating were carried out simultaneously rather than sequentially.

The purpose of the present study was to determine whether there is an effect of temperature on radiosensitivity of an animal virus, since the previous work had been confined to bacterial viruses.

MATERIALS AND METHODS

Test virus. The virus used was Newcastle disease virus (NDV). Allantoic fluids harvested 48 to 72 hr after infection of 10-day-old chicken embryos were pooled, centrifuged at 8,500 rev/min for 20 min, and stored at -40 C in 15-ml portions until used.

Irradiation of samples. The irradiation was carried out with gamma rays from a Mark I United States Atomic Energy Commission Cobalt-60 Food Irradiator. Under the conditions of the experiment, the dose rate, as determined by ferrous-ferric dosimetry, was approximately 4,850 rad/min.

Three-milliliter quantities of the infective allantoic fluid were filled into glass tubes (8 by 150 mm). For temperature control during irradiation, the tubes were placed in a stainless-steel jacketed vessel containing water tempered to the desired temperature. Constant temperature was maintained by circulating properly tempered water through the jacket. The entire apparatus was contained within an insulated steel chamber which was lowered into the gamma ray field for the prescribed time.

The assay method for hemagglutinin and infectivity was reported in the preceding publication (3).

RESULTS AND DISCUSSION

In Fig. 1 are presented the curves for inactivation of NDV infectivity by irradiation at various temperatures ranging from 2.2 to 60 C (36 to 140 F). The logarithm of the survival fraction \( V/V_0 \) has been plotted as a function of the irradiation dose in kilorads. It is of interest to note that for irradiation at the low temperature the inactivation curve was linear, whereas the inactivation curves for irradiation at the higher temperatures were nonlinear, consisting of a fast-inactivating component followed by a slower-inactivating component. This same pattern characterized the thermal inactivation curves for NDV infectivity (3). This result may indicate that the inactivation of NDV infectivity by simultaneous irradiation and heating was due principally to the thermal effects with the irradiation augmenting the effect of heat. The inactivation rate during
irradiation at lethal temperatures is the integral sum of three different components, that is, the effect of irradiation, the effect of heat, and the effect of an interaction between irradiation and heat if one is present. On the assumption that these three effects are additive, the interaction between heat and irradiation in the inactivation of NDV infectivity was determined by subtracting the slopes of the heat inactivation curves (3) from the slopes of the radiation inactivation curves for the same temperature. The radiation inactivation rates thus corrected for heating effect were plotted as a function of irradiation temperature (Fig. 2). Since both the thermal inactivation curves and the irradiation-heating curves were two-component types, two curves are shown in Fig. 2, one representing the primary slopes (fast-inactivating component) and the other representing the secondary slopes (slower-inactivating component). In both cases, it was indicated that there was no effect of irradiation temperature on inactivation of NDV infectivity over the temperature region of 2.2 to 49 C, but at temperatures greater than 49 C there was a marked increase in radiosensitivity. The sharp increase in radiosensitivity that occurred in the temperature region of 40 to 50 C has also been reported for T1 bacteriophage (1) and Salmonella typhimurium (9). The following theory has been postulated for the synergism between thermal and ionizing energy. The loss of biological function of some macromolecule, such as protein, by thermal denaturation requires the rupture of at least three adjacent bonds, such as hydrogen bonds, which causes the molecule to open up and lose its biological configuration. Rupture of one of these bonds by irradiation would lessen the requirement for the number of bonds to be broken by the thermal energy (1).

One may take an alternative approach to this problem and correct the irradiation-heating curves for the component effect caused by irradiation. This was done in the present study, and again the interaction effect was indicated at temperatures greater than 49 C.

In 1953, Epstein (4) proposed that the radiosensitive matter of virus is the nucleic acid and this has since been confirmed. However, Wilson and Pollard (11), by using a technique of charged particle bombardment with carefully controlled penetration, deduced that the radiosensitive volume for NDV is about 20% of the whole virus. Since the total ribonucleic acid (RNA) content of the virus (about 5%) would be expected to occupy less volume than this, they proposed that some of the radiosensitive material includes protein.

In the present study, it was found that an irradiation dose of 1.25 × 10⁶ rad at 2.2 C had no effect on the hemagglutinating property of NDV. Yet an irradiation dose of this magnitude at a temperature of 2.2 C affected a 7.5 log₁₀ reduction in infectivity of the virus. Thus, at low temperatures of irradiation, the hemagglutinin of NDV was more radioresistant than the infectivity property. Therefore, it can be concluded that inactivation was due principally to damage to the RNA. This finding has also been reported for other animal viruses (6, 10, 12). However, it is believed that loss of NDV infectivity by irradiation at lethal temperatures was mainly due to protein denaturation, although some RNA degradation may have also occurred. This supposition is predicated on the result that, when an Arrhenius plot of the radiation inactivation rate constants was made, a two-component curve was obtained. The break in the curve occurred at a temperature between 37.8 and

---

**Fig. 1. Inactivation of Newcastle disease virus infectivity at various temperatures by gamma radiation.**

**Fig. 2. Effect of irradiation temperature on inactivation rate of Newcastle disease virus infectivity.**
43.3C. This would indicate that two different mechanisms of inactivation were involved over the temperature range investigated.

ACKNOWLEDGMENTS

We thank E. R. Pfefferkorn of the Harvard Medical School for supplying the Newcastle disease virus.

This investigation was supported by Public Health Service grant EF-00006-09 from the Division of Environmental Engineering and Food Protection.

LITERATURE CITED

1. Adams, W. R., and E. Pollard. 1952. Combined thermal and primary ionization effects on a bacterial virus. Arch. Biochem. Biophys. 36:311–322.

2. Buzzel, A., and M. A. Lauffer. 1952. X-ray studies on T4 bacteriophage. Arch. Biochem. Biophys. 39:195–204.

3. DiGioia, G., J. J. Licciardello, J. T. R. Nickerson, and S. A. Goldblith. 1970. Thermal inactivation of Newcastle disease virus. Appl. Microbiol. 19:451–454.

4. Epstein, H. T. 1953. Identification of radiosensitive volume with nucleic acid volume. Nature (London) 171:394.

5. Gowen, J. W., and A. M. Lucas. 1939. Reaction of variola vaccine virus to roentgen rays. Science (Washington) 90:621–622.

6. Jaggar, J., and E. C. Pollard. 1956. Inactivation with fast charged particles of infectivity and hemagglutination in influenza A virus. Radia. Res. 4:1–19.

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

8. Lea, D. E. 1947. Action of radiations on living cells. Cambridge Univ. Press, New York.

9. Licciardello, J. J. 1964. Effect of temperature on radiosensitivity of Salmonella typhimurium. J. Food Sci. 29:469–474.

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

11. Wilson, W., and E. Pollard. 1958. Radiation studies on the infective property of Newcastle disease virus. Radia. Res. 8:131–141.

12. Woese, C., and E. C. Pollard. 1954. The effect of ionizing radiation on various properties of Newcastle disease virus. Arch. Biochem. Biophys. 50:354–367.