Growth of Vaccinia Virus in X-Irradiated Chick Embryo Tissues as Studied in Tissue Culture.* (21174)

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The effects produced by X-rays on innate and acquired immunity of the intact animal have been reviewed(1). In general, the results have been variable, depending on the host-infectious agent system employed and the dosage, time and method of administration of X-radiation. More recent reports (2-5) have shown that prior exposure of the whole animal to X-rays increases its susceptibility to selected viral agents. This effect may be due to an impairment of the antibody response as shown by Dixon *et al.*(6). The use of isolated host cells propagated *in vitro* rather than the intact animal would eliminate many of the immunological factors present in the latter. This paper reports the effects of X-rays on chick embryonic tissue as demonstrated by changes in its ability to support the growth of vaccinia virus in tissue culture. Since this agent in common with other viruses is an obligate intracellular parasite, radiation effects produced in the host cell might be reflected by changes in the amount of viral activity present in the fluid and/or tissue phases of tissue cultures.

Materials and methods. Skin, muscle, and cartilage tissues obtained from 6-14-day-old developing chick embryos were used. The technics of preparing and of incubating roller tube cultures as used in this study followed those described by Feller *et al.*(7). Nutrient fluid containing lactalbumin hydrolysate medium as described by Melnick and Riordan (8) and supplemented with 0.5% chick embryo extract was employed. Penicillin and streptomycin were added to give a final concentration of 50 units/ml and 50 γ/ml, respectively. The strain of vaccinia virus was obtained from Sharp and Dohme, Inc., in the form of calf lymph smallpox vaccine and was carried through 4 serial passages on the chorioallantoic membrane of 12-day chick embryos in our laboratory. Titrations for viral activity were made employing the standard technic(9). Statistical analysis of our titration results showed that the mean of the sample lay within ±20% of the true mean approximately 95% of the time.

Method of radiation. X-radiation was administered as a single massive dose to the tissues in roller tubes or to the chick embryo *in ovo*. The roentgen rays were generated by 2 electrically rectified X-ray apparatuses whose factors were: 1) 100 kv, 4 m.a., filtered through 1 mm Al (HVL 1.53 mm Al), target distance 30 cm delivered at dosage rates of 15.4 and 17.0 roentgens (r) per minute in air as measured through glass (Kimble borosilicate) and egg shell, respectively; 2) 150 kv, 20 m.a., filtered through 0.5 mm Cu plus 1 mm Al (HVL, 0.46 mm Cu), target distance 17 mm delivered at a dosage rate of 102 r per minute in air as measured through the egg shell.

Experimental procedures and results. In the first experiment a comparative study was made of the growth curves of vaccinia virus in tissues which had been exposed to varying amounts of X-rays. Tissue cultures were prepared from 8-day-old chick embryos and incubated in the roller drum for 3 days. At the end of this period, fluids were removed and tissues irradiated employing dosages of 500 r, 1,000 r, and 1,500 r (delivered at 15.4 r per minute). A fourth set of tissue cultures which were not exposed to X-rays served as a control. Fresh nutrient fluid was added to all the tubes and the tissue cultures incubated for 24 hours. At this time, the tissue cultures were inoculated with 2.0 ml of nutrient fluid containing vaccinia virus (3,000 infectious par-

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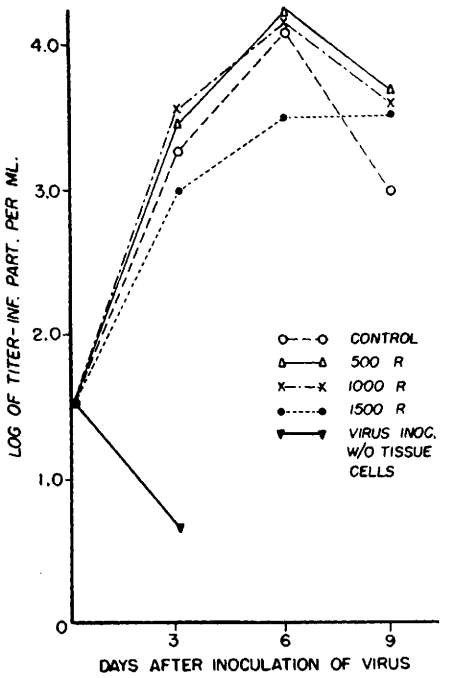

FIG. 1. Growth curves of vaccinia virus in chick embryonic tissue culture exposed to varying dosages of X-radiation.

ticles/ml). As additional controls, uninoculated sets of irradiated and normal tissue cultures were included. Nutrient fluids were replaced at 3-day intervals and the harvested materials titrated in duplicate for viral activity. The results (Fig. 1) showed no significant differences in infectivity titers of the irradiated and normal tissue cultures, with one possible exception. As compared to normal tissue cultures those irradiated with 1,500 r showed a suggestively lower infectivity titer at the end of 6 days.

In the second experiment the effect of serial passage of the virus in normal and irradiated tissue cultures was studied. The virus was carried in parallel through 5 serial passages in normal chick embryo tissues and in similar tissues irradiated with 500 r. The other conditions were similar to those described for the preceding experiment except that tissue cultures were incubated for 5 days, the fluids harvested, diluted 1:50 in fresh nutrient fluid and used as inoculum for the next passage. Infectivity titrations performed on 5th passage material yielded titers of 140,000 and 250,000 infective particles per ml, respectively for irradiated and unirradiated tissues.

In the third experiment the virus was *serially passed* in tissue cultures prepared from chick embryos which had been irradiated with 250 r X-rays *in ovo*, administered at 17.0 r per minute. To this end, 8-day-old embryos were irradiated and reincubated for an additional 24 hours. Tissue cultures were then prepared and incubated for 24 hours. Thereafter, the cultures were inoculated with vaccinia virus and carried through 3 serial passages in parallel irradiated and unirradiated tissue cultures. Conditions of incubation and of serial passage were the same as for Exp. 2. The third passage fluids were titrated and found to contain 850 and 250,000 infective particles per ml, respectively for irradiated and unirradiated tissues.

In the fourth experiment the effects of varying the ages of embryos used and the time of planting tissue cultures after exposure of tissues to irradiation were studied. Embryos 6, 10, and 14 days of age were used. The time intervals between exposure to irradiation and planting of tissue cultures were 0, 24, and 48 hours. Conditions of irradiation as well as planting, incubation and harvesting of fluids were similar to Exp. 3. The inoculum used to infect the tissue cultures had an infectivity titer of 3,000 infective particles per ml. Harvested fluids were titrated and the results are shown in Table I.

In the fifth experiment a study of 2 additional variables, *viz.*, total X-ray dosage (250

TABLE I. Multiplication of Vaccinia Virus in Tissue Cultures Prepared from Chick Embryos of Varying Ages Irradiated (250 r) *In Ovo* and Planted at Stated Time Intervals after Exposure.

Time interval between X-radiation and planting of tissue cultures (hr)	Exp. No.	Age of embryos (days) 6	10	14
0	1	108,000*	18,500	8,000
	2	77,000	15,000	8,000
24	1	28,000	14,800	19,000
	2	34,000	13,200	21,500
48	1	52,500	8,600	11,500
	2	32,500	7,000	6,200
Control (unirradiated tissues)	1	134,000	35,000	9,000
	2	92,000	32,500	11,250

* Infective particles/ml of tissue culture fluid.

TABLE II. Multiplication of Vaccinia Virus in Chick Tissue Cultures Prepared from Embryos Irradiated *In Ovo* with Varying Dosages of X-rays Delivered at Stated Dosage Rates.

Dosages (r)	Exp. No.	Dosage rates (r/min.) 17.0	102.0
250	1	52,000*	42,500
	2	45,000	46,600
500	1	35,500	14,000
	2	48,500	16,000
Unirradiated cultures	1	126,000	
	2	142,000	

* Infective particles/ml of tissue culture fluid.

r, 500 r, and 1,000 r) and rate of administration (17.0 r per minute and 102 r per minute) was carried out. Six-day-old chick embryos were irradiated *in ovo* and allowed to incubate 24 hours. The embryos receiving 1,000 r failed to survive the latter period and were therefore discarded. The remaining embryos were used to prepare tissue cultures. Control cultures were planted employing unirradiated tissues. Both sets of cultures were incubated for 24 hours, inoculated with vaccinia virus and then incubated for 5 days. Fluids were harvested and titrated for viral activity. Results are presented in Table II.

In the sixth experiment the growth curves of the virus in the fluid and tissue phases of irradiated and unirradiated tissue cultures were studied. Six-day embryos were irradiated *in ovo* with 250 r. The chick embryos were incubated 24 hours and then harvested and tissue cultures prepared from normal and irradiated tissues. After an additional 24-hour incubation period both sets were inoculated with virus. Fluids were replaced every 2 days in those cultures which were incubated beyond that period. At intervals beginning with one hour and continuing through 15 days, cultures were harvested. Infected fluids were processed as in previous experiments. Infected tissues were triturated with sterile alundum to which 0.9 ml of Hanks balanced salt solution was added per tissue culture as diluent. The supernatant was removed after centrifugation (2,400 rpm for 20 minutes) and considered to be a 10^{-1} dilution. Titrations for viral activity were performed in duplicate and results used as a basis for Fig. 2.

Discussion. Our experimental results have indicated that the exposure of chick embryo cells propagated in tissue culture to single doses (500-1,500 r) of X-radiation did not affect the ability of these cells to support the growth of vaccinia virus. In contrast, tissue culture cells derived from chick embryos which had been exposed *in ovo* to a smaller dose (250 r) of X-rays 24 hours prior to explantation showed a significant reduction in their ability to support the growth of the virus. These differences in the effects produced by *in ovo* and *in vitro* irradiation suggest that the diminished ability of tissues irradiated *in ovo* to support viral growth was due chiefly to the secondary effects of radiation rather than to primary ones. Further evidence supporting this view is obtained from observation that tissues explanted to tissue culture immediately after exposure to X-radiation *in ovo* were less affected than those irradiated *in ovo* 24 hours prior to explantation. This interval would not enhance the primary effects of radiation but would leave the cell exposed to the influence of secondary radiation effects for a longer time. The significant differences in the mode of action of primary and secondary radiation effects has been reviewed by Lea(10).

The growth patterns of vaccinia virus in chick embryo tissue irradiated *in ovo* and in normal tissue were quite similar. The only

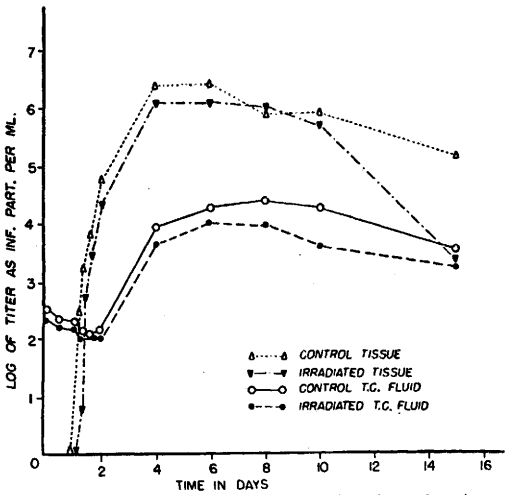

FIG. 2. Growth curves of vaccinia virus in tissue culture prepared from normal and *in ovo* (250 r) X-irradiated chick embryo.

significant difference noted was the markedly lower viral titers obtained in the irradiated tissue cultures. Although direct evidence is lacking it is likely that this diminished ability of cells to support viral multiplication is due to cellular damage resulting from radiation exposure, as has been described in the case of a bacteriophage-X-ray inactivated bacteria system(11). That diminished viral multiplication may be a result of decreased cellular proliferation is suggested by the observation that normal tissue cultures derived from 10-14-day chick embryos yielded lower viral titers than did those derived from younger (6-day) embryos.

The diminished susceptibility of cells irradiated *in ovo* to support viral multiplication is in contrast to reports stating that the radiation of the intact animals results in an increase in susceptibility to viral infection. In another virus-host-cell system utilizing tissue culture technics, we have been unable to overcome the innate resistance of embryonic and adult cells to viral infection by means of exposure to ionizing radiations. Attempts to propagate the MEF1 strain of Type 2 poliomyelitis virus in tissue culture employing mouse embryonic skin and muscle fibroblast-like cells and adult mouse kidney epithelial-like cells previously exposed *in vitro* or *in vivo* to X-rays or beta rays have been unsuccessful.

Summary. The effect of X-radiation of chick embryonic tissue has been studied in regard to its subsequent ability to support the multiplication of vaccinia virus in tissue cultures. Under the stated experimental conditions radiation of the tissue *in vitro* had no significant effect; in contrast, exposure of the embryonic tissue to radiation *in ovo* appeared to diminish its ability to support viral growth in tissue culture. The differences noted were fairly uniform throughout the period studied. Evidence suggesting that the differences noted are due chiefly to secondary radiation effects is discussed.

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A New Count of Allantoic Cells of the 10-Day Chick Embryo. (21175)

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Numerous viruses and some other parasites are often propagated in the cells lining the allantoic cavity of the developing chick embryo. In the case of influenza and some other viruses, many quantitative and kinetic investigations on problems relating to virus reproduction have been carried out with allantoic cells, usually in eggs inoculated at the 10th day of incubation. In order to compute the ratio of adsorbed particles to available cells and the yield of virus particles per cell, it is necessary to know the number of cells lining the allantoic cavity.

This number has been estimated by several indirect methods and by direct count of the nuclei in a preparation, and the various estimates agree fairly well(1). However, recent data obtained in this laboratory indicated that