Table 1. Lack of base sequence homology between MS2- and Q_β-RNA as shown by annealing experiments. The ribonuclease sensitivity of mixtures of P^{32}-labeled MS2- or Q_β-RNA with unlabeled MS2- or Q_β-specific double-stranded RNA was determined after thermal denaturation and reannealing. Conditions: P^{32}-MS2-RNA, 0.15 μg; P^{32}-Q_β-RNA, 0.24 μg; MS2-specific double-stranded RNA, 8.7 μg; Q_β-specific double-stranded RNA, 6.2 μg. Heating for 3 minutes at 120°C in 0.02 ml of 2.5 × SSC was followed by annealing for 60 minutes at 85°C. Sensitivity to ribonuclease was measured (*13*).

Annealing mixture		Acid-insoluble radioactivity (count/min)	
Type of P^{32}-RNA	Type of double-stranded RNA	No ribonuclease	Ribonuclease
Experiment 1			
MS2	MS2	9,900	8209
MS2	Q_β		45
MS2	None		32
Experiment 2			
Q_β	Q_β	11,375	9050
Q_β	MS2		120
Q_β	None		45

determine the proportion of radioactive plus and minus strands in each synthetase product, the C^{14} radioactivity was plotted against P^{32} radioactivity, and the slope and intercept of the resulting straight line (Fig. 3) were computed by the least-squares method. The slope corresponds to the fraction of C^{14} radioactivity in plus strands while the intercept gives that of C^{14} radioactivity in minus strands (*13*). Three closely agreeing experiments with the MS2 and two with the Q_β synthetase product showed that the former contained an average of 75 percent plus and 25 percent minus strands, whereas the latter had 85 percent plus and 12 percent minus strands.

Phage RNA replication occurs in two steps (*14*): (i) synthesis of complementary minus strands, with viral plus strands as template, resulting in the formation of a virus-specific replicating complex and (ii) synthesis of viral progeny plus strands with the minus strands of the replicating complex as template. Although the two steps are genetically separable (*16*), no separate enzymes for each step have yet been isolated. Spiegelman's RNA replicase preparations (*17*), which specifically require homologous viral RNA for activity and synthesize infectious RNA (*18*), catalyze both steps. During the first few minutes of incubation of Q_β replicase with radioactive nucleoside triphosphates, there is predominant synthesis of minus strands, despite reports to the contrary (*19*); later on, plus strands are synthesized in much larger amounts (*12*). One of the main differences between replicase and synthetase preparations is apparently that the former are free of RNA primer and require the addition of homologous RNA, whereas the latter contain viral minus strands (possibly protein-bound), and require no addition of template. Furthermore, in the initial phase of the reaction, Q_β replicase produces almost exclusively viral minus strands which are then presumably used as template, for the synthesis of Q_β-RNA, whereas synthetase produces predominantly plus strands from the outset of the reaction.

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1. Abbreviations: RNA, ribonucleic acid; SSC, 0.15*M* sodium chloride, 0.015*M* sodium citrate; "plus" strands are the viral parental-type strands; "minus" strands are those with a complementary base sequence; UTP, uridine triphosphate.

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Oncogenicity by DNA Tumor Viruses: Enhancement after Ultraviolet and Cobalt-60 Radiations

Abstract. *Simian virus-40, polyoma, and LLE46 virus preparations were treated with ultraviolet or gamma radiations (cobalt-60) in a frozen state. Infectivity and induction of complement-fixing antigen and DNA synthesis declined as a logarithmic function of dose, the latter two properties being more resistant than infectivity to radiation by a factor of 2 to 5. Oncogenicity of all three viruses did not decrease with progressive amounts of both types of irradiation, but actually increased in absolute and relative terms (per infectious unit), even at the maximum dose of irradiation used (24,000 ergs per square millimeter per minute and 2.7 × 10^6 rads).*

Some functions of the genome of tumor viruses are more resistant to physical or chemical inactivation than the function of replication. Thus, the induction of complement-fixing antigens by polyoma, SV40, and LLE46 (a hybrid of adenovirus 7 and SV40) viruses (*1*), the induction of DNA synthesis by SV40 and polyoma (*1, 2*), the induction of thymidine kinase by SV40 (*3*), and the transformation of cells by polyoma (*4, 5*) can be dissociated from the capacity to produce infectious progeny. It is conceivable that the oncogenic interaction of the virus with the target cells requires the expression of only a limited number of functions of the viral genome.

We now report the increase rather than decline of tumorigenicity in vivo per infectious unit of LLE46, polyoma, and SV40 after ultraviolet or cobalt-60 irradiation.

The method of irradiation has been reported (*1*). Briefly, for Co^{60} irradiation, viruses, after separation from cellular debris, were suspended in 1 percent tryptone solution at a final concentration of 10^7 to 10^8 $TCID_{50}$ (tissue culture infective doses, 50 percent effective) or plaque-forming units (PFU) per milliliter. Irradiation was performed in a Co^{60} irradiation source at the Pennsylvania State University (by W. Ginoza).

In order to minimize secondary

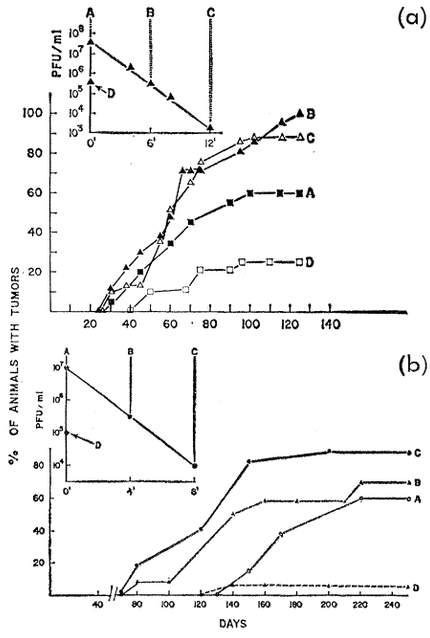

Fig. 1. Effect of ultraviolet treatment on infectivity and tumorigenicity of (a) PV and (b) SV40. Rate of tumor induction of nonirradiated and ultraviolet-treated viruses. Inserts indicate the rate of loss of infectivity (ordinate) in function of time of irradiation (abscissa); the letters identify the irradiated samples inoculated into hamsters.

effects during irradiation, virus preparations were frozen in dry ice or liquid nitrogen. At various times, samples were removed and kept at −70°C until time of testing. For ultraviolet irradiation, the virus suspensions were spread in a thin layer in 150 mm petri dishes and exposed to an ultraviolet germicidal lamp (Westinghouse Sterile lamp, 782-L-20). Infectivity of LLE46 was tested in culture tubes of human kidney; SV40 was titrated by plaque assay in green monkey kidney or CV-1 cells; and polyoma, by plaque assay in secondary cultures of mouse embryo cells. Litters of Lakeview hamsters were inoculated subcutaneously with 0.1 ml of virus preparations 24 to 36 hours after birth.

Infectivity of the three viruses declined as a logarithmic function of dose, whether they were treated with ultraviolet light or with gamma radiations (Fig. 1, a and b; and Tables 1 and 2).

The rate of inactivation of induction of complement-fixing antigen and DNA synthesis was also of the first-order kinetics, but was more resistant to radiation by a factor of 2 to 5, according to the virus tested (*1*), than infectivity was.

Tumorigenicity, however, presented a completely different picture. Inactivation of all three viruses by either method did not decrease their ability to induce tumors, even at a radiation dose sufficient to reduce the infectivity titer of the irradiated samples by a factor of 10^3 to 10^4. A concentration of the nonirradiated virus which would give less than 100 percent tumor incidence was selected. Tumorigenicity per infectious unit actually increased in the irradiated virus samples, as demonstrated in experiments in which the untreated virus was diluted to an infectious titer equivalent to that of the ultraviolet-irradiated samples (Table 1). In all cases, the median latency time for tumor appearance was shorter with the irradiated than with the untreated viruses.

The untreated LLE46 virus did not produce tumors, while the ultraviolet-irradiated sample was tumorigenic even when its infectivity titer was reduced by a factor of 10^4 (Table 1).

The histopathological features of several tumors from the various series examined were similar to those of tumors produced by untreated virus. In addition, 35 tumors from the SV40 and LLE46 irradiation experiments were tested by immunofluorescence for specific SV40-induced complement-fixing antigen. All had a proportion of positive cells ranging from 60 to 100 percent at the first passage in vitro.

The finding that virus inactivation actually enhanced tumorigenesis was unexpected because polyoma virus-transformation of cells in vitro decreases with increased doses of radiation (*4*, *5*). There is, however, precedence for this phenomenon. Increased tumorigenesis of mammary tumor viruses after gamma radiation has been reported by Moore (*6*) and by Ardashnikova and Spasskaia (*6*, p. 107); similar results have been observed with ultraviolet and x-ray radiation of Friend leukemia virus (*7*). Because no independent test for infectivity was available, the increased tumorigenicity was the only viral function measured by these authors. Thus, it was suggested that irradiation destroyed an inhibitor of larger "target" size than the infectious tumor virus itself. This interpretation is not applicable to our experiments, since decay of infectivity and other viral functions, but not of tumorigenesis, occurs with progressive doses of radiation.

Another possibility is suggested by the work of Latarjet *et al.* (*5*, *8*) who have observed that Schmidt-Ruppin sarcoma virus, which survives a moderate amount of irradiation (320 to 950 kiloroentgens), may have an enhanced ability for virus synthesis. This does not appear to be the case in our system, however, because neither the rate of plaque appearance nor the diameter of the plaques formed by the irradiated viruses differed from that of the con-

Table 1. Effect of ultraviolet irradiation on infectivity and tumorigenicity of polyoma, SV40, and LLE46 viruses. The source of ultraviolet irradiation was a Westinghouse Sterile lamp, 782-L-20, delivering 95 percent of energy at 2537 Å. The virus samples (5 ml in a 150-mm petri dish) were placed 21.9 cm from the lamp. At this distance, the energy at surface is 2×10^3 erg/mm² per minute (calculated). Litters of Lakeview hamsters were inoculated within 2 days after birth and examined weekly for tumor appearance. Data are included from two different experiments for each virus preparation.

Irradiation (min)	Titer of inoculum	Incidence of tumor*	
		Number	Percent
	Polyoma virus		
0	$10^{6.5}$	35/58	60
0	$10^{4.6}$	6/23	26
6	$10^{4.5}$	49/49	100
12	$10^{2.4}$	45/49	92
	SV40		
0	10^{7}	8/13	62
0	10^{5}	3/39	8
4	$10^{5.5}$	12/18	67
8	10^{4}	15/17	88
	LLE46		
0	10^{7}	0/16	0
4	$10^{5.1}$	3/21	14
8	10^{3}	3/17	18

* Ratio of the number of hamsters with tumors to the number inoculated.

Table 2. Effect of Co^{60} irradiation on infectivity and tumorigenicity of SV40 and LLE46 viruses. Virus samples in 1 percent tryptone were maintained at −70°C in one experiment and in liquid nitrogen in the other during irradiation.

Dose (rad)	Titer of inoculum	Incidence of tumor*	
		Number	Percent
	SV40		
0	$10^{6.8}$	18/27	67
1.3×10^6	$10^{5.8}$	21/25	84
2.7×10^6	$10^{4.8}$	11/16	69
	LLE46		
0	$10^{6.6}$	9/24	37
6.6×10^5	$10^{5.3}$	10/22	45
1.3×10^6	10^{5}	13/20	65
2.0×10^6	$10^{4.4}$	17/23	74
2.7×10^6	$10^{3.7}$	19/32	59

* Ratio of the number of hamsters with tumors to the number inoculated.

trols. Furthermore, several experiments seem to indicate that, at least in the case of polyoma virus in hamsters, the interaction leading to tumor formation occurs within 24 hours after inoculation, and further viral replication, if it occurs, does not play any significant role in tumorigenesis (*9*). Thus, the increased frequency of tumors with irradiated viruses must be related to the properties of the virus population at the time of inoculation.

The possibility that irradiations could have modified those properties of the virus coat, which conceivably would favor adsorption of the virus to the target cells, can be excluded, since there is no enhancement of infectivity or of ability to produce complement-fixing antigen by the irradiated viruses. Furthermore, hemagglutination activity of polyoma virus—a property of the viral coat—was not affected by the maximum amount of ultraviolet radiation used (*10*).

Because ultraviolet and gamma radiations inactivate biological material through different mechanisms of action, the fact that both radiations produced enhanced tumorigenicity in three different viruses suggests nonspecificity of locus of action. At present we can propose two alternative hypotheses for the mechanism of increased oncogenicity of irradiated DNA viruses, both compatible with established experimental evidence.

1) Oncogenicity is a property of "defective" virus particles, and, in irradiated samples, because of inactivation of particles capable of expressing all the genome functions, the proportion of "defective" particles increases. As a further hypothesis it could be suggested that the fragments of viral DNA responsible for oncogenesis may be more easily incorporated into the cell genome if the integrity of the whole viral DNA is altered by radiation.

2) The tumors produced by the irradiated viruses are defective for the viral-induced specific transplantation antigens (*11*) and, consequently, their growth is not hampered by immunological interaction with the host. None of the tumors produced by the irradiated viruses that were examined was defective for the SV40-induced complement-fixing antigen.

Apart from the mechanism involved, these findings suggest the need for cautious use of inactivated tumor viruses for vaccine purposes and suggest that other viruses (for example, reoviruses, herpes, pseudorabies) be tested after suppression of their cytocidal properties by irradiation for possible unmasking of oncogenic potential.

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Tubular Structures Associated with Turnip Yellow Mosaic Virus in vivo

Abstract. *Plants infected with a necrotic strain of turnip yellow mosaic virus contain tubes averaging about 80 millimicrons in diameter and attaining 3 microns in length. The main constituent of these tubes is a protein that is related chemically and immunologically to the protein of the virus. The tubes are composed of hexagonally packed hexagonal subunits resembling the hexamer protein subunits of the virus particles and are probably helical in construction.*

Electron-microscopic examination of negatively stained crude extracts (*1*) of plants infected with a necrotic strain (*2*) of turnip yellow mosaic virus showed that infection led to the production of tubular structures, in addition to the near-spherical particles of the virus. The tubes, which flatten when dried for electron microscopy, varied in diameter between 30 and 130 mμ and were up to 3 μ long. Amino acid analysis of purified preparations of the tubes provided strong evidence that they are composed of viral protein. This conclusion was supported by demonstration of a serological relation between the virus and the tubes. In electron micrographs these tubes showed a somewhat confused appearance, although the presence across them of sets of evenly spaced lines suggested that regular fine detail had been resolved. The clear optical transforms of the tubes, formed with an optical diffractometer described elsewhere (*3*), confirmed this and indicated that the construction of the tubes was based on a hexagonal lattice with a periodicity of about 42Å. A typical transform is shown in Fig. 1. Doubling of the spots and their disposition indicate that the transform was produced by two superimposed identical lattices intersecting at a small angle and set symmetrically on either side of the long axis of the tube. This is a situation that would arise if the tube were helical in construction and, as can occur with negatively stained objects (*4*), had both upper and lower surfaces resolved in the electron micrograph. Since the lattices on the two sides of the tube are not in register, mutual interference between structural detail on each side would be expected and would explain the disordered appearance of the tube. The high degree

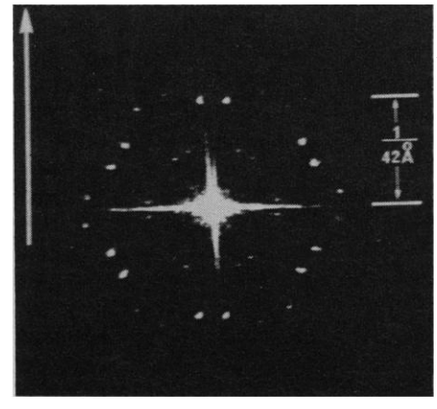

Fig. 1. Optical transform of the turnip yellow mosaic virus shown in Fig. 2. An arrow indicates the corresponding orientation of the long axis of the tube.