*RELATIVE TARGET SIZES FOR THE INACTIVATION OF THE TRANSFORMING AND REPRODUCTIVE ABILITIES OF POLYOMA VIRUS**

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Two kinds of virus-cell interaction occur in cells infected in tissue culture by polyoma virus: a cytocidal interaction accompanied by virus multiplication in mouse embryo cells,[1, 2] and a noncytocidal interaction in hamster cells resulting in the acquisition by the cells of permanently altered growth characteristics akin to those of malignant cells.[3, 4] The latter process, referred to as transformation, is generally regarded as the *in vitro* counterpart of the process whereby polyoma causes tumors in animals. It is not known whether all of the genes present in the polyoma virus DNA are required for transformation. This problem has now been studied by comparing the inactivation rates, or target sizes, of polyoma virus in regard to its cytocidal (plaque-forming) and transforming effects. Four different methods of inactivation have been used—ultraviolet and X irradiation, nitrous acid, and P^{32}-decay. With each of these agents, the target size of the transforming ability was found to be 55–65 per cent that of the cytocidal ability.

Materials and Methods.—The small-plaque variety of polyoma virus was grown and purified according to Winocour.[5]

Assays: The standard-plaque assay on mouse embryo cells was used.[6] Transformation was measured on BHK (baby hamster kidney) cells by using the agar suspension method.[7]

Inactivations: Procedures for UV and X irradiations are described elsewhere.[8] Total emission from a germicidal lamp was used as the UV source. The dose rate corresponds approximately to 50 lethal hits per min to bacteriophage T2 in the absence of photoreactivation. X rays (220-kv) filtered through 1 mm of aluminum were used at a dose rate of approximately 3250 roentgens per min. Nitrous acid inactivation was carried out by mixing equal volumes of 2 *M* $NaNO_2$, 1 *M* acetate buffer pH 4.5, and virus suspension. The reaction was allowed to proceed at room temperature for the times indicated, then stopped by rapid cooling and addition of an equal volume of cold 5 *M* Tris buffer pH 8.5, followed by dialysis against standard saline citrate buffer. P^{32}-labeled virus was prepared as follows: confiuent baby mouse kidney cultures were kept in phosphate-free medium for 24 hr before infection. The cultures were infected at a multiplicity of 10–50 PFU/cell, washed free of unadsorbed virus, and then incubated in Eagle's medium containing 10^{-5} *M* phosphate and 5 per cent dialyzed horse serum. RDE (receptor destroying enzyme) and antipolyoma antiserum were added to the cultures from 6 to 10 hr after infection to remove residual cell-associated virus. The cultures were again washed and reincubated in the same low phosphate medium. At the 14th hr after infection, carrier-free P^{32}-orthophosphate was added in an amount sufficient to make the specific activity of the medium approximately 1 P^{32}:200 P^{31}. The cultures were harvested at the 50th hr, the cells sonicated to release the virus, and the debris removed by low-speed centrifugation. The free P^{32} was removed by dialysis, and the

virus stored at −70°C for periodic assay. Unlabeled virus prepared under the same conditions, except for the omission of the isotope, was used as a control. To compensate for variations in the sensitivities of the assays from week to week, the survival values were taken as ratios of the titer of labeled to that of unlabeled virus as determined in the same assay.

Results and Discussion.—In Figure 1 *a–d* are presented typical survival curves for the plaque-forming and transforming abilities of polyoma virus for each kind of inactivation. In each case, the inactivation is a single-hit exponential process. The change in slopes of the UV-inactivation curves at higher doses is not important for the present discussion; its nature will be discussed elsewhere.[8] Only the initial slopes are considered here. For any of the methods of inactivation, the ratio of the slopes of the survival curves is a direct measure of the relative target sizes of the virus for the two effects. In repeated experiments, the values for this ratio (transformation:plaque formation) were always between 0.54 and 0.66 and, within these limits, did not depend on the kind of inactivation.

The consistently observed difference in target size suggests that the amount of viral genome required for transformation is approximately 55–65 per cent of the amount required for virus reproduction. The results, however, do not bear on the question of viral genes common to both types of interaction, i.e., the extent to which the two targets overlap. The interpretation in terms of the relative amounts of viral genetic material required for the two effects rests on two basic assumptions: (1) the DNA of the virus is the target of inactivation in each case, and (2) the probabilities of survival of viral function are not differentially modified by interaction with the two host cell species used.

The first assumption seems justified for the following reasons: (1) the extracted viral DNA has both plaque-forming[9] and transforming[10, 11] abilities; (2) the rates of UV inactivation of the plaque-forming ability of the virus and of the viral DNA are indistinguishable;[8] (3) the doses of X rays used here do not affect the hemagglutinating ability of the virus which is a function of the viral protein; and (4) the primary effect of P^{32}-decay is restricted essentially to the DNA which contains most, if not all, of the isotope.

The major uncertainty in the interpretation stems from the use of different cell species in the two assays. The results could be influenced by two classes of cellular factors: (1) Enzymatic repair of the lesions in the polyoma virus DNA. Such repair is known to be extensive for UV damages,[12] much less in the case of X rays,[13] and is not known for either nitrous acid[12] or P^{32}-decay. The ratios of the target sizes as determined by the various agents, however, are the same; therefore, it is unlikely that such repair plays any role; (2) Differences in the "code dictionaries" between mouse and hamster cells. Such differences would be relevant only if the induction of conditional lethal mutations[14] is an important mechanism of inactivation, and if some of these mutations are lethal in mouse but not in hamster cells. This could occur in virus inactivated by nitrous acid, UV, or X rays, which are known to be mutagenic, although mutagenesis is unlikely to be a significant cause of inactivation in the case of X rays. The possibility of P^{32}-decay acting in this way, however, seems altogether remote, since the decay is not known to be mutagenic and inactivates the virus presumably by causing double-stranded breaks in the DNA. Evidence against a differential effect by the host cells was also obtained in an experi-

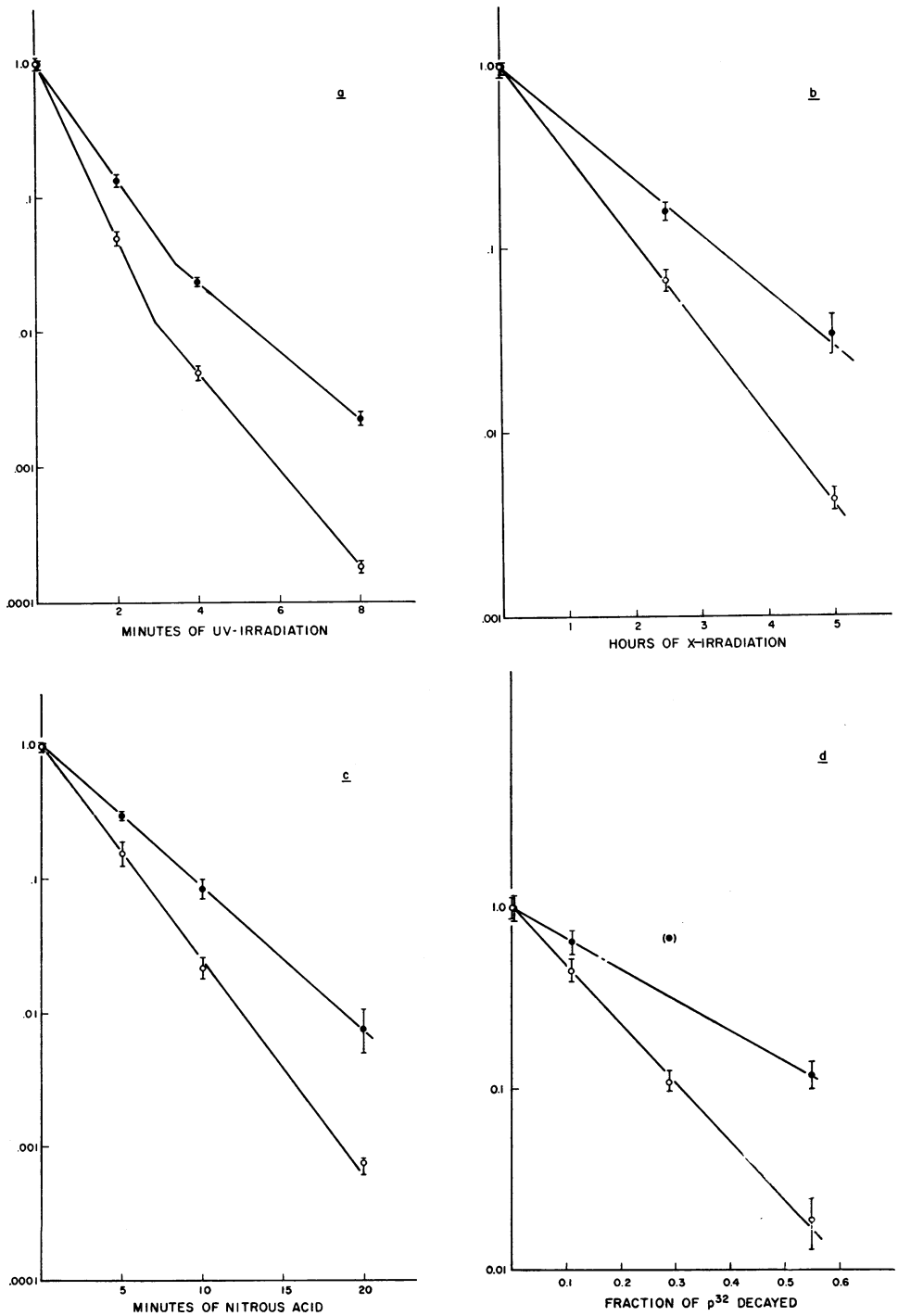

FIG. 1.—Inactivation of plaque-forming (○) and transforming (●) abilities of polyoma virus by (*a*) ultraviolet light, (*b*) X rays, (*c*) nitrous acid, and (*d*) P^{32}-decay. The logarithms of the surviving fractions are plotted on the ordinate, and the dose or treatment on the abscissa. The brackets around each point indicate 95% confidence limits.

ment in which the survival of the reproductive ability of polyoma virus DNA was measured in the small proportion of hamster cells in which a cytocidal interaction occurs. Hamster and mouse embryo secondary cultures were infected with unirradiated or UV-irradiated viral DNA, and the cells replated as infective centers on mouse embryo secondary cultures. The infected cells were treated just prior to transfer with a dose of X rays sufficient to destroy their ability to divide without affecting their capacity to support virus multiplication. With a single dose of UV to the polyoma virus DNA, the fractional survival was 0.36 ± 0.09 on mouse embryo cells, and on two preparations of hamster embryo cells 0.39 ± 0.07 and 0.33 ± 0.1. Since both enzymatic repair and mutational inactivation are perhaps most likely in UV-treated virus, this result shows that these phenomena do not play a major role in the target size determinations.

Multiplicity reactivation could conceivably contribute to the difference in inactivation rates, particularly in view of the higher virus to cell ratio required for transformation. This could not be a major factor, however, because in both assay systems the dose-response curves of virus inactivated by the various agents were linear.

Summary.—The rate of inactivation of the transforming ability of polyoma virus has been found to be 55–65 per cent of the rate of inactivation of the reproductive ability using four different methods of inactivation. These results have been interpreted to mean that transformation requires the participation of only 55–65 per cent as much of the viral DNA as does plaque formation.

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