Radiation Carcinogenesis at Low Doses

Abstract. An analysis of experimental findings indicates that the induction of a mammary neoplasm in the Sprague-Dawley rat is dependent on the action of radiation on more than one cell. Although a linear relation between incidence and x-ray dose might be consistent with available data, such a relation would be fortuitous and linear extrapolation to lower doses is unjustified.

Carcinogenesis induced at low radiation doses is a problem of crucial importance to modern radiation technology. Organizations responsible for recommending values of the maximum permissible dose (MPD), such as the International Commission on Radiological Protection and the National Council on Radiation Protection and Measurements, have stated that "prudence" requires the assumption that the frequency of harmful effects is proportional to dose at low doses (1). Although these organizations have made it plain that this assumption is intended to provide a conservative estimate of the upper limits of incidence (2), calculations based on this assumption have been performed to predict that there would be a large number of cancers induced in the population of the United States each year if it were to be exposed near the MPD levels (3). Bond has pointed out that exposure near the MPD of even a large fraction of the population is virtually impossible and also that the incidence frequencies employed in these calculations are probably many times too large (4). These factors might reduce the calculated incidence by 100-fold or more, but adherence to linear extrapolation indicates nevertheless a finite incidence of cancer at all doses. This reasoning would also imply the need for rigorous control of the exposure of individuals to radiation for medical purposes and might even induce attempts to minimize exposure to natural background radiation which would represent the principal source of radiation carcinogenesis in the population.

It has not been possible thus far to check the validity of the "prudent assumption" of linearity between dose and effect by direct observation at absorbed doses of x-rays of less than several rads. However, we present here some pertinent conclusions that can be drawn on the basis of data obtained at higher doses in cases where radiations of high linear energy transfer (neutrons) have been experimentally applied. Such conclusions will be presented here. Although the analysis does not provide a definitive answer to the basic question of whether there exists a "threshold dose" for cancer induction, it may serve to clarify some of the issues involved.

The absorbed dose is delivered by individual charged particles, that is, in discrete statistically independent events of energy deposition. The spectrum of energy deposition events is independent of the magnitude of the dose. From this fact one can conclude (5) that in the limit of very low doses any effect is proportional to dose if it can be produced by a single particle. If n particles are involved in the induction of the effect, the dose-effect relation must be proportional to the nth power of the dose when the dose is small (that is, if the frequency of particles in the volume of interest is much less than unity). Even if n is only 2, a linear downward extrapolation by a factor of 10 will, under these conditions, introduce an overestimate by a factor of 10 since the incidence, instead of being 10 times less, will in fact be 100 times less.

The question of whether single particles are involved in the production of neoplasms can be analyzed in the case of mammary neoplasms of the Sprague-Dawley rat. Like most other malignancies of interest in radiation research, these tumors are atypical since they occur with appreciable frequency in control animals. In addition, less than half of these tumors are malignant. Nevertheless, these data have been used to obtain estimates of cancer incidence at low doses, and there are various aspects of the following discussion that should apply to carcinogenesis in general.

Figure 1 shows data by various authors (6-9) on the incidence of neoplasms as a function of the dose of x-rays (200- to 250-kv energy) and "fission" neutrons (10). The following discussion is concerned with the mechanism of tumor induction at low doses. The data points in the range of high doses where tumor incidence reaches a maximum value and subsequently declines (6, 9) are therefore not included in Fig. 1. The various studies appear to be in substantial agreement, and, in the case of x-rays, a linear dependence of incidence on dose appears to be consistent with the data, but linearity seems less consistent with the results for neutrons.

This conclusion becomes much more evident when the data points are plotted on a logarithmic representation where linearity should result in lines with a slope of 1. Such a representation is shown in Fig. 2, where the mean number of tumors induced per animal is plotted instead of the fraction of animals with tumors. The two quantities coincide at small incidences; at larger doses the number of tumors per animal has been taken from the original work (6) or, where not observed experimentally (7-9), has been deduced from the fraction of animals with tumors. The correction for multiple tumors has been based on the assumption that the tumors occur independently (see below). The spontaneous incidence has been subtracted. Neither set of data appears to be consistent with linearity. However, a logarithmic plot of the ratio of doses for equal incidence (the relative biological effectiveness) versus the x-ray dose approximates lines with slopes of -1 at low doses (Fig. 3). This is in accord with previous observations (5) and with the theory of dual radiation injury (11).

According to this theory, lesions produced by a single neutron secondary particle (usually a proton) require the action of two x-ray secondary particles (electrons). Hence in the range covered in Fig. 3 the primary lesions underlying the effect must have predominantly a quadratic dependence on x-ray dose. This does not eliminate the possible existence of a weak linear component for radiation of low linear energy transfer that might become
dominant at x-ray doses that are much lower than those covered in Figs. 1–3. It must, however, be concluded that, even if the dose-effect relation for x-rays were in fact linear in the dose range investigated (a distinct possibility in view of the limited accuracy of the experiments), the linearity must be accidental and there is no justification for a linear extrapolation.

The complexity of the process is apparent because there is no linear relation for neutrons, since at low doses the slope in the logarithmic representation is less than 45 degrees. Indeed, at first sight, it would appear that the assumption of linearity is not conservative but instead underestimates the hazard at low doses since the incidence increases with a power of the neutron dose that is less than unity.

Microdosimetric determinations (12) have shown that it requires a dose of somewhat over 20 rads of fission neutrons to achieve an average of one traversal through cell nuclei having a diameter of about 7 \( \mu \)m. The neutron dose-effect curve extends well below this radiation dose, and near its lower range the probability of one neutron secondary particle per nucleus and even per cell approaches 0.1. This result, however, does not imply that individual cells are the foci of carcinogenesis. On the contrary, the fact that the dose-effect relation deviates from linearity at a dose where the mean number of neutron secondary particles in a cell is considerably less than unity implies that the development of malignancies must be determined by radiation effects on a number of interacting cells. It may be helpful if some explanatory remarks precede the formal proof of this assertion.

It has been generally observed that biological variability of radiosensitivity flattens the dose-effect curve; whenever the more sensitive subgroups of the population are exhausted by nearly full incidence, the logarithmic slope of the dose-effect relation decreases. One might therefore assume that variance of sensitivity between animals or between cells in individual animals may explain the observed dose-effect relations.

An analysis of the frequency of multiple tumors (6) shows that this frequency follows Poissonian statistics, which eliminates the possibility that the incidence of one tumor decreases the induction probability of further malignancies in the animal. It is therefore possible to correct the original data which represent the fraction of animals having at least one tumor and to derive

\[
E(D) = \sum_{r=1}^{\infty} E_r e^{-\phi D} \frac{\phi D^r}{r!}
\]

where \( n = \phi D \) is the mean number of charged particle traversals at dose \( D \), that is, the expectation value of the event number \( v \).

From this general formula one can deduce without further assumption that the slope in the double-logarithmic representation must always be at least equal to \( (1-\phi D) \). This result is derived as follows:

\[
\frac{d \ln E(D)}{d \ln D} = \frac{D}{E(D)} \frac{dE(D)}{dD} = \frac{D}{E(D)} \sum_{r=1}^{\infty} E_r \left[ e^{-\phi D} \frac{\phi D^r}{(r-1)!} \right] \frac{\phi e^{\phi D}}{v!} = \frac{1}{v!} \frac{\phi}{E(D)} \sum_{r=1}^{\infty} E_r \left[ e^{-\phi D} \frac{(\phi D)^r}{r!} \right]
\]

By use of Eq. 1 and a rearrangement of terms one obtains:

\[
\frac{d \ln E(D)}{d \ln D} = \frac{\sum_{r=1}^{\infty} E_r e^{-\phi D} \frac{(\phi D)^r}{v!} (v-\phi D)}{\sum_{r=1}^{\infty} E_r e^{-\phi D} \frac{(\phi D)^r}{v!}} = (1-\phi D)
\]

or

\[
\frac{d \ln E(D)}{d \ln D} = \frac{\sum_{r=1}^{\infty} E_r (\phi D)^r (v-\phi D)}{\sum_{r=1}^{\infty} E_r (\phi D)^r} \geq (1-\phi D)
\]
Morphological Transformation in vitro of Human Fibroblasts by Epstein-Barr Virus: Preliminary Observations

Abstract. Human embryo fibroblasts have undergone morphological transformation in vitro after infection by Epstein-Barr virus. The fibroblasts were maintained in suspension during exposure to the virus, and further treatment with inactivated Sendai virus increased the transformation rate. The transformed cells were large and polygonal and grew in discrete, heaped up, foci.

Since the first discovery of Epstein-Barr (EB) virus in cultured Burkitt lymphoma cells (1), evidence implicating this agent as a possible cause of the tumor has steadily grown. Thus, the virus stimulates human lympho-proliferation both in vitro (2) and in vivo (3) and is linked with the Burkitt lymphoma both on seroepidemiological grounds (4) and because the tumor cells carry virus-determined surface neoantigens (5) as well as the viral genome (6).

With a suspected human tumor virus, there are great difficulties in devising experiments to show conclusively that the suspect virus in fact plays an etiologic role in a particular malignant disease. Accordingly, it was considered that at the experimental level new information on the oncogenic potential of EB virus might be obtained if some conventional demonstration of in vitro cellular transformation could be achieved. Although it has long been known that EB virus cannot be made to infect any of a wide variety of monolayer test tissue cultures by standard techniques (7), it was thought that some special manipulation might allow infection to take place. Experiments have therefore been...