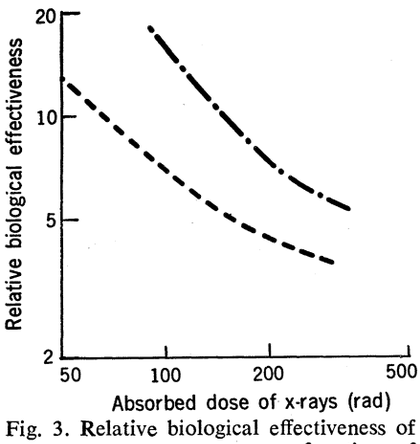

Fig. 3. Relative biological effectiveness of neutrons versus x-rays as a function of x-ray dose. The curves are derived from the interpolated lines in Fig. 2 [(- - - - - -) Shellabarger (*8*); (—. —.) Vogel (*7*)].

The conclusion that the slope is greater than or equal to $(1-\phi D)$ follows from the fact that the numerator in Eq. 4, contains the same positive terms as the denominator, each term being multiplied by the factor $(\nu-\phi D)$ which is always at least as large as $(1-\phi D)$. This result proves the theorem.

As stated above, ϕD is of the order of 0.1 at the lowest doses of fission neutrons. The assumption of unicellular origin for the malignancy then requires the slope of the logarithmic dose-effect line to be no less than 0.9. Since the slope is, in fact, 0.5 or less, it must be concluded that in the dose range investigated the carcinogenetic process cannot reflect radiation injury to individual cells in a population of noninteracting cells. This statement applies even if these cells have an arbitrary distribution of sensitivities and if the "carcinogenesis" consists in the induction of a variety of neoplasms.

The process must therefore involve energy absorption by more than one cell, and the situation is too complicated to warrant a linear extrapolation to low doses. To account for the shallow slope in the region investigated, it is necessary to assume that radiation absorption events can both promote and inhibit tumor induction which thus must involve more than one kind of process as well as more than one cell.

As mentioned earlier, the frequency of multiple tumors (*6*) appears to follow a Poissonian distribution; in particular, the maximum fraction of animals with only one tumor is very close to 37 percent, as would be expected if there are no interactions between tumors. The inhibitory effect deduced can therefore not be one exerted by the presence of other tumors. The carcinogenetic action seems, in fact, to be controlled by local phenomena, and the process has been termed "scopal," not only because tumors were induced only in the portion of the animal irradiated but also because they have been induced with apparently equal efficiency when small pieces of mammary tissue were irradiated in vitro and then reimplanted (*13*). Although hormonal and immunological factors controlling tumor development have been identified when whole animals are exposed (*14*), there seems to be no known evidence for local radiation-induced inhibitory action.

With the complexity of the tumor induction process established, there remains little justification for linear extrapolations, and this conclusion, in turn, removes apparent inconsistencies between the dose-effect relation and postulates (*15*) and histological evidence (*16*) to the effect that carcinogenesis requires the transformation of several contiguous cells. There is, however, at present, insufficient evidence for numerical estimations of tumor incidence based on linear or other extrapolations.

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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 etiological 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

undertaken in which human embryo fibroblasts were mixed, not as a monolayer, but in suspension with partially purified EB virus for 1½ hours to facilitate viral adsorbtion. After this, some of the cells were further exposed to inactivated Sendai virus in order to assist entry of adsorbed EB virus by the induction of cell fusion.

The EB virus in the inoculums was prepared (courtesy of the John L. Smith Memorial for Cancer Research, Maywood, New Jersey) from P3HR-1 Burkitt lymphoblasts (*8*) by Sharples centrifugation and filtration through a Millipore (0.8 μm) filter; electron microscopy indicated that this material contained 3×10^7 virus particles per milliliter. Sendai virus, at a concentration of 20,000 hemagglutinating units per milliliter in Earle's balanced salt solution (EBSS), was inactivated by exposure to ultraviolet light for 30 minutes at 15 cm from a 15-watt lamp (Englehard Hanovia). For the experiments, second passage human embryo skin and muscle fibroblasts were trypsinized and suspended in Eagle's basal medium with 5 percent fetal calf serum.

The EB virus suspension (2.5 ml) was added to fibroblasts (4×10^6) in 3 ml of the medium, and the mixture was kept in suspension at 37°C for 1½ hours by rotation on a Matburn cell mixer (Baird and Tatlock) at 25 rev/min. The cells were then deposited by centrifugation at 260*g* for 5 minutes, washed by suspending and depositing three times in fresh medium, and resuspended in 1 ml of EBSS with 30 percent fetal calf serum. Inactivated Sendai virus (2.5 ml) was added to this cell suspension which was then placed at 4°C for 30 minutes; the mixture was then rotated at 25 rev/min on the Matburn mixer at 37°C for 1 hour. The cells were washed three times as before, and resuspended in Eagle's basal medium with 10 percent fetal calf serum at a concentration of 2×10^5 cell/ml, and distributed in 30-ml plastic culture bottles (Falcon) to give a total of 10^6 cells per bottle, Control cell suspensions were set up in the same way, except that exposure to either EB virus or Sendai virus was omitted. All bottles were gassed with 5 percent CO_2 in air, incubated at 37°C, given a change of medium every 5 days, and observed daily.

After a 7-day incubation period, the fibroblasts in all bottles had formed a confluent monolayer. Among the normal fibroblasts in all bottles exposed to EB virus, groups of 20 to 30 large polygonal cells were observed. In the bottles where cells were, in addition, exposed to inactivated Sendai virus after the EB virus, the number of such groups was about seven times greater. No polygonal cells were present among the fibroblasts treated only with the Sendai virus. After 9 days of incubation, the groups of polygonal cells had increased in size to form appreciable colonies (Fig. 1) which continued to grow. By 19 days, the colonies were macroscopically visible within the fibroblast cell sheet and were seen to be heaped up in the shape of a cone with a central crater (Fig. 2).

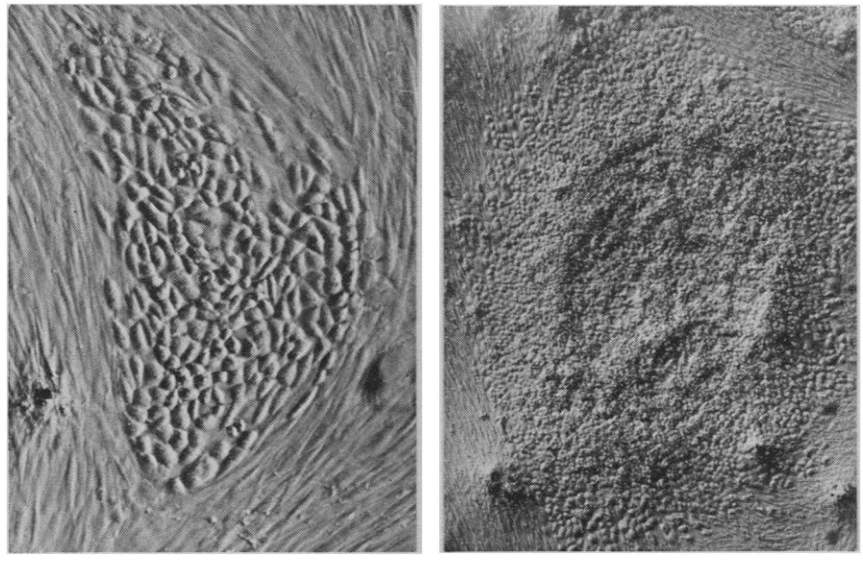

Fig. 1 (left). Photomicrograph of a living culture of human embryo fibroblasts 9 days after infection in suspension with EB virus. A colony of morphologically transformed polygonal cells can be seen within the sheet of normal fibroblasts. Oblique illumination (× 72). Fig. 2 (right). Similar preparation to that shown in Fig. 1, but taken 19 days after infection with EB virus. The morphologically transformed polygonal cells have proliferated to form a cone-shaped colony with a central crater. Oblique illumination (× 27).

Passage of picked colonies of polygonal cells gave rise to cultures in which such cells predominated over the fibroblastic elements that were, inevitably, also transferred; on further passage the polygonal cells outgrew the fibroblasts to give a uniform population. The polygonal cells apparently have the fine structural features of altered fibroblasts and are not releasing EB virus. In addition, the cells have a doubling time during exponential growth of 30 hours and show a plating efficiency in soft agar of about 14 percent. Karyotyping has indicated that they have normal human chromosomes with a near hexaploid mode.

The EB virus thus appears to have brought about morphological transformation of human embryo fibroblasts in vitro when infection was carried out with the cells held in suspension, and the transformation rate was appreciably increased where cell fusion was induced after adsorption of the infecting EB virus.

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