

Transplantation of Human Malignant Epithelial Cells from Tissue Culture to Rat Brains*

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SUMMARY

The rat brain has been shown to be a useful site for the transplantation of human, malignant cells previously grown *in vitro*. Although several lines of established cultures of epidermoid carcinoma cells (KB and three lines of HeLa) produced tumors after inoculation into the brains, a difference in the growth capacity of the various lines of cells was observed.

The occurrence of mucin production in the tumors was an unexpected finding, since this feature was not described in the biopsy specimens of the human neoplasms from which the KB and HeLa cells were originally obtained. Also, mucin was not demonstrated in the malignant cells *in vitro* in this laboratory.

Poliomyelitis virus multiplied in the tumor-bearing brains, but the cytopathogenic effect of the virus was not comparable to the pronounced destructive changes produced by the virus in the neoplastic cells *in vitro*.

In this laboratory, transplantations of tissues, organs, and cells from tissue culture are being carried out in rats, hamsters, chicks, and chick embryos. The main objective of these experiments has been to study the effects of the internal environment of a host on the multiplication of viruses in the transplants.

The present article is concerned with one aspect of the investigations: namely, the use of the rat brain as a site for the transplantation of human cancer cells previously grown in tissue culture. The cells used in this study were obtained from established cultures of two human epidermoid carcinomas. One of these was a cervical carcinoma from which Gey and associates (2) developed a serial culture passage *in vitro* since February, 1951 (7). The other was a carcinoma of the floor of the mouth from which a line of cells, designated KB, was established in tissue culture by Eagle (1) following initial planting of material from the original tumor in December, 1954.

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MATERIALS AND METHODS

Weanling Wistar rats weighing 40-60 gm. were conditioned by means of x-radiation and cortisone. Two days prior to cell implantation, the animals were given 328 r whole-body x-radiation; the next day they received 3 mg. of cortisone acetate. The day after transplantation a second injection of cortisone acetate was administered. Animals were killed, and the brains were removed at varying time intervals. Sections were made at five different levels anteroposteriorly, and each section was examined microscopically.

Three lines of HeLa cells, each obtained from a different laboratory, and KB cells were employed. HeLa (MBA), originally obtained from Microbiological Associates, Inc., and HeLa (D), furnished by Dr. E. H. Lennette, were used shortly after they were received. HeLa (T), supplied by Tuskegee Institute, was used after many passages in this laboratory, the earliest passage employed being No. 50. Near the end of the investigation, it was necessary to check certain results, so that new HeLa (T) cells were obtained from Tuskegee Institute and were used shortly after their arrival.

The cells were grown in Eagle's medium containing 10 per cent serum with 200 units of peni-

cillin and 100 μ g streptomycin/ml. Both human and calf sera were employed. The cells were passaged at approximately weekly intervals. For transplantation purposes, the cells were dislodged from the surface of the glass by treatment with trypsin, 0.06 per cent, at 37° C. The treatment usually required 15–20 minutes. The process of cell separation sometimes required gentle scraping with rubber policemen. The cell suspension was centrifuged at 600 r.p.m. for 10 minutes and resuspended in growth medium with 5 per cent serum, but containing no antibiotics, so as to yield 1.5×10^7 cells/ml. The cells were injected intracerebrally in 0.1-ml. amounts. This was done by means of a 25-gauge needle, inserted directly through the skin and skull, usually in the left parietal region, while the animals were anesthetized lightly.

RESULTS

Growth capacity of the malignant cells.—The several lines of established cultures of epidermoid carcinoma cells used in this investigation were observed to produce tumors after inoculation into brains of rats. The growths were found in the brain tissue, in the ventricles, and in the meninges, not only at the site of inoculation but at various levels of the brain. In most animals, the tumor was found in a majority of the sections studied, but sometimes it was limited to one section. The amount of tumor varied with the duration of time after inoculation and in accordance with the type of cells used.

The degree of growth was decided upon arbitrarily. It was considered *minimal* when only small nests of cells were identified microscopically in the various sites of the brain, and *abundant* when a fairly large area was replaced by tumor in one or more sections (Fig. 1). The designations *slight* and *moderate* were applied when the amount of tumor was considered to be between these two degrees.

Very little was identified on gross examination of the brains. Only rarely was a tiny piece of tumor evident on the surface of the brain. Occasionally, hydrocephalus of moderate degree was observed, even though no tumor was recognized grossly. In four instances, tumor masses were found in the subdural or epidural area, attached to the dura but not adherent to the brain itself.

The degree of growth of the different lines of cells, as observed microscopically, is indicated in Table 1. It will be observed that the KB line of cells and HeLa (MBA) had a tendency to develop abundant growth of tumor, for which reason they have been designated “high malignancy” lines. On the other hand, HeLa (T) produced only slight growth, behaving as a “low malignancy” line of

cells. The HeLa (D) cells have not been studied sufficiently, but they appeared to have the capacity to produce better growths than the low-malignancy line of HeLa. Quantitatively similar results were obtained with cells grown in the presence of either human or calf serum. The age of the cells ranging from 3 to 9 days after initiation of a culture did not influence the growth of tumor in the rat brain. There was no apparent difference in the one experiment in which the animals were conditioned by x-radiation alone.

Microscopic details of the growths.—The tumors consisted of masses or sheets of cells with no differentiation toward keratinizing squamous epithelium (Fig. 2). The cells were generally round, with moderate amount of granular cytoplasm. In the more

TABLE 1
THE DEGREE OF GROWTH OF VARIOUS LINES OF MALIGNANT CELLS TRANSPLANTED TO RAT BRAINS

Line of cells	Days after inoculation	Degree of growth	No. rats studied
KB	4	Slight	21
	6–8	Slight	13
	11	Moderate to abundant	64
	15–18	Abundant	10
HeLa (MBA)	4	Minimal	13
	6–8	Moderate	7
	11	Abundant	40
	15	Abundant	15
HeLa (T)	4	Minimal	14
	6–8	Minimal	51
	11	Slight	43
	15	Slight	56

abundant growths, the cytoplasm was clear in certain portions, giving the appearance of so-called “clear-cell” carcinoma. Sometimes along the periphery of the masses the cells were elongated and arranged in the form of a palisade, a feature which was seen more often in the KB tumors.

The nuclei, usually round or ovoid, showed considerable variation in size and staining quality. Mitotic figures, typical and atypical, were readily identified. Nuclear variability and enlarged, hyperchromatic nuclei were more prominent in the KB and HeLa (MBA) tumors than in the HeLa (T). However, even more pronounced nuclear pleomorphism, with gigantic and multinucleated forms, was strikingly evident in the HeLa (D) growths.

Necrosis of tumor tissue was noted, especially in some of the more abundant growths; thus it was seen more frequently in the KB and HeLa (MBA) tumors. As a rule, the necrosis was of the focal

type, with the individual, closely grouped cells of the involved areas showing chiefly karyorrhexis and pyknosis of nuclei and lysis of the cytoplasm, the latter often being transformed into a hyalin mass. Generally, there was no significant cellular response to the necrotic tissue even in the larger areas of involvement. Only seldom was an occasional neutrophil observed among the necrotic cells.

Mucin production.—In the more abundant tumors small cystic foci resembling glands were commonly noted (Fig. 3). These cystic areas contained a pale eosinophilic or basophilic material, which sometimes gave a staining reaction that is characteristic of mucin (namely, red with Mayer's mucicarmine, purple-red with periodic acid-Schiff method, and metachromatic with toluidine blue). Striking, also, was the presence of groups of cells in some of these tumors, not necessarily associated with the cystic areas, which were enlarged and had pale, finely vacuolated cytoplasm and often peripherally situated nuclei (Fig. 4). Mucin was demonstrated in these cells by the technics mentioned above.

Host reaction to the tumors.—A characteristic of the growths resulting from each of the lines of cells used in this study was the notable lack of response of the brain and meninges to them (Figs. 1–3). In only very few instances was there the slightest evidence of any tissue reaction. In these cases, there was an insignificant glial proliferation about some of the tumor masses in the brain tissue with only an occasional neutrophil, and in the vicinity of the meningeal tumors a few histiocytes and lymphocytes were observed. An exception to this was at the site of inoculation in the brain, where, regardless of whether tumor was present or not, there was a reaction that could be attributed to mechanical trauma produced by the needle; namely, necrosis and glial proliferation with typical "gitter" cell response. Varying degrees of hemorrhage and hemosiderin deposition were also evident at this site. In contrast, there was a pronounced desmoplastic reaction in the four tumors found in the subdural and epidural areas.

FIG. 1.—Section of rat brain showing tumor, 15 days after inoculation of HeLa (MBA) cells, infiltrating the corpus callosum and filling the lateral ventricles. Host reaction about the lesion is negligible. Hematoxylin and eosin. $\times 8.5$.

FIG. 2.—Portion of a HeLa (MBA) tumor in another rat brain to show details of its growth, its infiltration of the white matter without host reaction, and its presence in the ventricle. Hematoxylin and eosin. $\times 112$.

DISCUSSION

Heterologous and homologous tumors have been successfully transplanted into brains of animals (4–6, 8). Also, the rat brain has been employed as a site for the transplantation of normal monkey kidney cells previously grown *in vitro* (9). In the present study, it has been shown that this site is useful for the transplantation of human, malignant cells obtained from *in vitro* cultures.

Although others have been able to produce good growths in brains of animals not conditioned by such means as x-radiation or cortisone, we were unsuccessful in our earlier attempts to produce tumors after intracerebral inoculation of malignant cells from tissue culture in the nonconditioned host. A detailed investigation, however, was not made to determine whether other factors contributed to the failure in these experiments. More work along this line will be done.

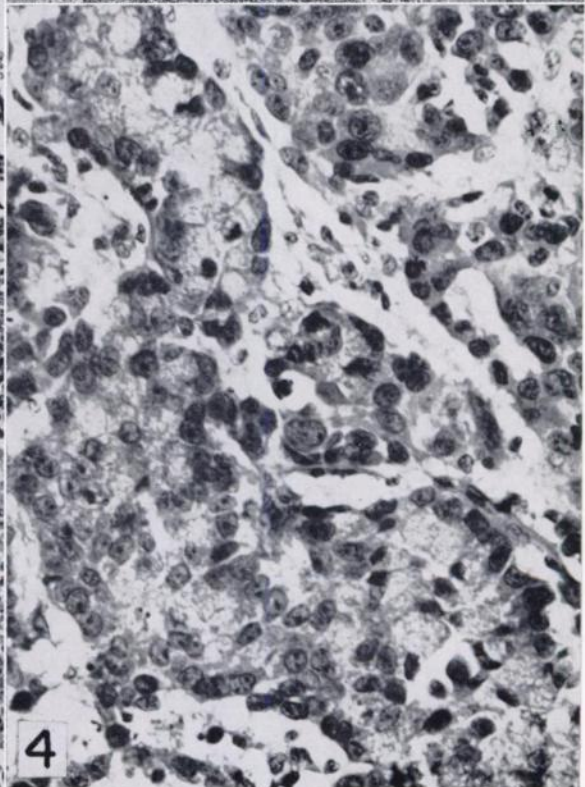
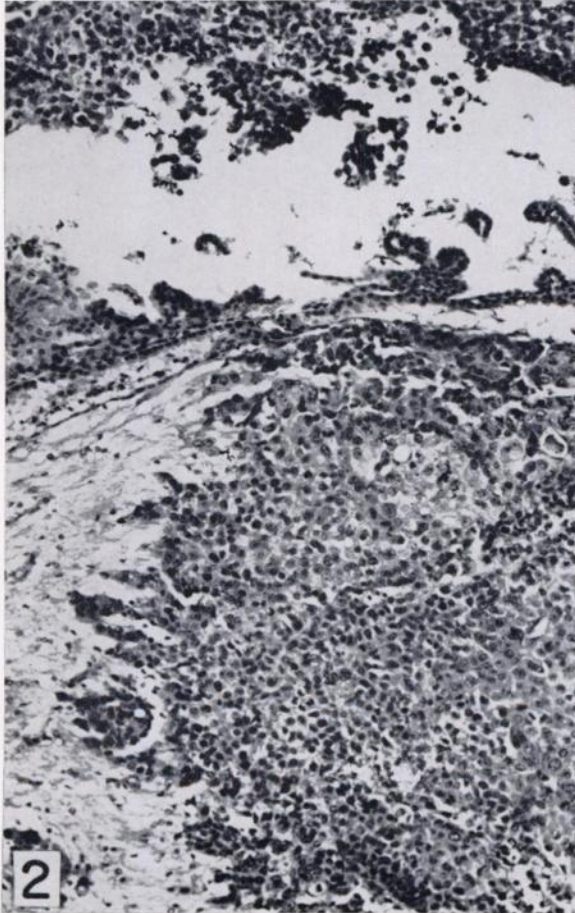
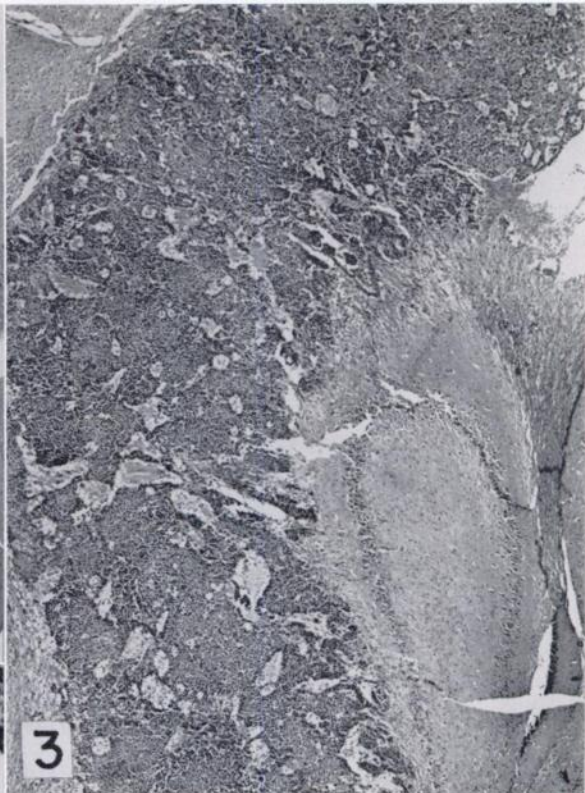
As pointed out by Greene (4), the major disadvantage of employing the brain as a site for heterologous tumor transplantation is the inability of the investigator to see or palpate the growing transplant. However, a significant advantage is the notable lack of host reaction in and about the developing tumors, except at the site where trauma was produced by insertion of the needle. The lack of reaction in the brain tumors is in contrast to the marked desmoplasia observed in the growths which developed in the subdural and epidural areas in a few of the animals in these experiments.

Multiple tumors were found in various areas and at different levels of the rat brains (in the meninges on the surface and in the sulci, in the ventricles, and in the brain tissue itself) because of the ready transportation of the malignant cells by the fluid of the ventricular system and of the subarachnoid space.

It has not been determined what factors are responsible for the different capacity of growth of the various lines of HeLa cells. At first, it was thought that the low-malignancy line, HeLa (T), had been modified during the many passages to which it had been subjected in this laboratory prior to its transplantation to rat brains. However,

FIG. 3.—Higher magnification of tumor in Figure 1 to show cystic spaces containing material which gave staining reactions that are characteristic of mucin. Hematoxylin and eosin. $\times 29$.

FIG. 4.—Another HeLa (MBA) growth in a rat brain to show groups of enlarged cells with finely vacuolated cytoplasm and often peripheral nuclei. These are mucin-producing cells. Hematoxylin and eosin. $\times 340$.



the new HeLa (T) cells, which were obtained near the end of this investigation and which were used shortly after their arrival in order to check the results, produced a similar pattern of growth. It appears, therefore, that some change may have occurred in this line prior to its receipt in this laboratory.

The production of mucin in the HeLa and KB tumors was an unexpected finding, since, apparently, this change was not observed in the biopsy specimens of the original epidermoid carcinomas. Mucin was not demonstrable in the malignant cells *in vitro*. This mixture of epidermoid and mucin-producing cells is reminiscent of that noted in the so-called mucoepidermoid tumors. It would be desirable to attempt to correlate this particular finding with various factors in the animal hosts. Of interest, in this regard, is the observation by Glücksmann (3) that there is a relationship between hormonal changes of pregnancy and the development of a type of cervical carcinoma with epidermoid and mucinous features which he calls "mixed carcinoma."

In a preliminary study in this laboratory (10), it was found that poliomyelitis virus multiplied in brains bearing tumors derived from HeLa and KB cells, but not in brains receiving no tumor cells. A relative increase of necrosis and partial inhibition of growth have been observed in some of the virus-infected tumors, but considerable portions of tumor remained unaffected. In some experiments, there was no increase of necrosis, compared with the controls, even though the virus reached adequate levels of multiplication. This is in contrast to the pronounced destructive changes produced by the virus in the tumor cells grown *in vitro*. Further investigation of this problem is under way.

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