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Specificity of the Control of Tumor Formation by the Blastocyst[1]

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ABSTRACT

An assay to determine the mechanism of regulation of embryonal carcinoma cells by the blastocyst, which is based on a comparison of tumors produced when the cancer cells are cloned alone or after incorporation into blastocysts, was refined by labeling embryonal carcinoma cells with fluorescent microspheres and by following their fate after injection into the blastocysts. Through the use of the new techniques, it was observed that cells of one line of nullipotent embryonal carcinoma were controlled at the 50% level, those from another were not controlled, and those from a multipotent but undifferentiated line were controlled in almost absolute fashion.

Single Sarcoma 180 or L1210 leukemia cells were not controlled when injected into the blastocele, but C1300 neuroblastoma cells were partially controlled. None of these tumors have a normal cellular counterpart in the blastocyst, as does embryonal carcinoma, but neurulation follows blastulation by only a few days, so that the neuroblastoma cells may be regulated at that time. Parietal yolk sac carcinoma cells, which have a counterpart in the late blastocyst, were not controlled.

On the basis of these data, it is postulated that, if one embryonic field can regulate its closely related cancer, then there may be an embryonic field capable of regulating each carcinoma.

INTRODUCTION

Directing the differentiation of malignant to benign cells as an alternative to cytotoxic therapy for metastasizing carcinomas has been a dream of this laboratory since the first demonstration that some of the progeny of embryonal carcinoma cells could spontaneously differentiate into benign cells (12, 21, 22). Subsequently, differentiation has been observed in a wide variety of neoplasms, and it has been induced by irradiation in some tumors (9) or by a variety of chemicals in others (7, 31). Although these therapies have elicited important scientific information, they are often impractical for clinical use because they lack specificity in their effects and are usually toxic for normal cells. Retinoids have enhanced endodermal differentiation in embryonal carcinoma cells (33). Other approaches are to use known mediators of differentiation or extracts of embryos in attempts to direct differentiation or to inject tumor cells into embryos and observe the effects on tumor production (6, 27–29, 34).

Although we were able to enhance muscle differentiation in long-term tissue cultures of embryonal carcinomas (25), attempts to direct differentiation of embryonal carcinoma using various tissues and organ extracts during the 1960's failed. In 1974, Brinster (5) demonstrated that the blastocyst of the mouse was capable of regulating an embryonal carcinoma cell. In these experiments, an embryonal carcinoma cell of a strain of mouse of one coat color was injected into the blastocyst of a strain of mouse of another coat color. The injected blastocysts were transferred to the uteri of animals made pseudopregnant. One of the mice that was born had coat colors reflecting a dual origin, one color indicating the expected contribution of cells from the blastocyst and the other indicating a contribution from the cancer cells. Clearly, the blastocyst could regulate embryonal carcinoma cells and their progeny to the point that they no longer behaved in malignant fashion; rather, they participated in normal embryonic development resulting in functional mice. The Brinster experiment was confirmed by Mintz and Illmensee (16) and by Papaioannou *et al.* (18). The former demonstrated the proportion of cancer-derived cells in selected tissues of the chimera, and the latter demonstrated that there was an upper limit of cells that could be controlled by the blastocyst. If as many as 20 embryonal carcinoma cells were injected into the blastocyst, then a mouse was born that had tumors.

We developed an assay to define the mechanism whereby the blastocyst regulates embryonal carcinoma cells and their progeny (23). Embryonal carcinoma cells lose tumorigenicity immediately upon differentiating; the assay was based on a comparison of the incidence of tumors produced from single embryonal carcinoma cells injected into animals either alone or after incorporation in blastocysts. We found that the blastocyst could control a single embryonal carcinoma cell of strain 402A$\bar{x}$ provided that that cell was placed in the blastocele. If placed between the zona pellucida and trophectoderm (the perivitelline space), it was not controlled. The blastocyst could not control 3, 4, or 5 402A$\bar{x}$ cells, nor could it control a B-16 melanoma cell (23).

It would appear that the cancer cells, which are the neoplastic equivalent of the inner cell mass cells of the blastocyst, are controlled by embryonic signals or by the environment of the inner cell mass and then take part in embryonic development. Since B-16 melanoma cells are not directly analogous to any of the cell types of the blastocyst, there is no reason to expect the blastocyst to control a melanoma cell.

We now report further experiments upon the ability of the blastocyst to control tumorigenicity of lines of embryonal carcinoma with marked improvements upon the technology used. In addition, other experiments testing the specificity of blastocyst control of cancer have been performed. While the embryonal carcinomas 402A$\bar{x}$ and 247 were controlled by the blastocyst, F-9 was not. Sarcoma 180, parietal yolk sac carcinoma, and L1210 leukemia were not controlled by the blastocyst, but there was a degree of control of C1300 neuroblastoma.

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MATERIALS AND METHODS

The tumors used, including source, host strain, diagnosis, means of routine propagation, cloning site, and efficiency, are listed in Table 1. 402Aẍ and F-9 have been designated nullipotent because they do not form the differentiated tissues diagnostic of teratocarcinomas when propagated s.c. or in the intraperitoneum (3). Some of their cells synthesize basement membrane and have other features of immature endoderm (30). They have been maintained in strain 129 mice although Isa and Sanders have demonstrated that 402Aẍ will grow in all strains of mice (11). None of the tumors cloned in the subcutaneum, some cloned in the intraperitoneum, and some only in the testes (Table 1). Line 247 is a rapidly growing tissue culture line developed from OTT6050 which, although extremely undifferentiated, forms few tissues when grown in the s.c. space (13). It is near diploid. This tumor clones at the 10% level in the testis, an incidence considered to be too low for the assay. Tumors can be grown from 5 cells implanted in the testis in 30 to 40% incidence.

Blastocysts were flushed with balanced salt solution containing 3 mg bovine serum albumin per ml from Swiss-Webster mice at 3.5 days of pregnancy. These blastocysts formed benign teratomas in testes of strain 129 animals in 10% of cases (23). These small tumors never contained embryonal carcinoma or formed ascites. When tumors developed in testes from blastocysts which were given injections of malignant cells, the testes were replaced by masses of malignant tissue of the same type as that of the injected cell. Benign teratomas were never observed in any of the histological sections of these tumors. When blastocysts were placed i.p., they were destroyed by a homograft rejection (23). The immunological rejection of the Swiss-Webster blastocyst by the homologous host did not prevent formation of tumors by embryonal carcinoma cells, which were isologous with the host, because tumors in comparable incidence were produced from cells cloned in the absence of blastocysts or after incorporation into the perivitelline space of the blastocyst (23).

Suspended tumor cells were obtained as follows: (*a*) ascites cells were washed once with balanced salt solution and used immediately; (*b*) cells in tissue culture were suspended by standard trypsinization techniques, washed once, and used immediately; (*c*) cells from solid tumors were minced and explanted overnight in tissue culture, and adherent tumor cells were suspended by trypsinization prior to use. Cells were tested for viability using trypan blue exclusion and were placed with the appropriate number of blastocysts in a small drop of growth medium (Eagle's minimum essential medium with 10% fetal calf serum) in a 60-mm plastic Petri dish. The drop was covered with washed mineral oil. The techniques for injection were as reported previously. Briefly, a blastocyst was attached by gentle suction to a holding pipet, which was made from capillary tubing on a vertical pipet puller. Single cells were aspirated into fine injecting pipets that were used either blunt (1) or after sharpening on a diamond wheel (15), and the cell was injected into the blastocele using a Leitz micromanipulator (Fig. 1).

Injected blastocysts were transferred to the appropriate site in an animal, and single cells were injected into the same anatomical site in control animals. Injected animals were maintained for 2 months, but most of the tumors developed after about 30 days, and none developed after 6 weeks. All solid tumors were examined histologically to confirm the diagnosis. The results of each experiment were expressed as a frequency of tumor formation. For each series of experiments with a particular cell line, the results were compared to those for the control animals by the statistical method of Mantel and Haenszel (14), which is an extension of the χ^2 test.

Early in the course of these experiments, 402Aẍ, which was used as a control in this series of experiments on specificity, had extremely variable results. Accordingly, we refined the techniques to eliminate technical error. To this end, embryonal carcinoma cells were labeled with polystyrene beads (fluorescent monodispersed carboxylated microspheres measuring 0.7 μ; Polysciences, Inc.) by incubating 4×10^5 embryonal carcinoma cells for 16 hr in 2 ml of growth medium containing 4×10^8 microspheres. The cells phagocytosed enough beads to be identified with a fluorescent microscope yet not enough beads to interfere with clonability.

Two important sources of error were found. The first was to believe that the labeled cell had been placed in the blastocele when, in fact, the tip of the injecting pipet was separated from the cavity by a thin membrane of inverted trophectodermal cytoplasm (Fig. 2*a*). This artifact, which resulted in placement of the cell in the perivitelline space, was obviated by observing the mode of exodus of the labeled cell from the tip of the injecting pipet. The second source of error was the escape of the labeled cell from the blastocele either to the perivitelline space or from the blastocyst (Fig. 2*b*). This artifact was minimized by reducing the pressure within the blastocele after the injection of the cell and prior to the removal of the pipet. As a result, there was little or no flow of fluid to carry the cell from the blastocele. Leakage of cells from blastocysts which were given injections of single cells was reduced by these means to less than 10% of cases. In the case of Tumor 247, 5 cells were injected into blastocysts, and the leakage approached 50%.

Injected blastocysts usually collapsed into a ball of cells surrounded by the zona pellucida. Rather than inject these collapsed blastocysts into animals immediately, as we had done in the past, they were incubated *in vitro* for 1 hr to allow the blastocele to reform. These blastocysts were then rolled around with a needle attached to the micromanipulator to facilitate microscopic identification of the appropriate number of tumor cells in the blastocyst (Fig. 3). The cancer cells were easily distinguished from the embryonic cells by their refractibility (Fig. 4) and by their polystyrene beads (Fig. 5). Aliquots were taken

Table 1

Tumor and origin	Host strain	Methods of propagation	Cloning site and efficiency	Comments
402Aẍ embryonal carcinoma [Isa and Sanders (11)]	129	Ascites	Intraperitoneum (20–40%)	Ascites, typical small vesicles; nullipotent
F-9 embryonal carcinoma [Rizzino and Sato (26)]	129	Subcutaneum	Testis (30–40%)	Solid tumor, grown *in vitro* for 16 hr prior to experiment; nullipotent
247 embryonal carcinoma [Lehman *et al.* (13)]	129	Monolayer tissue culture	Testis (15%)	Maintained as clean embryonal carcinoma by rapid passage; multipotent but undifferentiated
L1210 leukemia (Animal/Human Tumor Bank)	DBA/2	Ascites	Intraperitoneum (25–35%)	Ascites
Sarcoma 180 (Animal/Human Tumor Bank)	DBA/2	Ascites	Testis (20–25%)	Ascites
C1300 neuroblastoma (Jackson Laboratory)	A/j	Subcutaneum	Testis (40–50%)	Solid tumor, grown *in vitro* for 16 hr prior to experiment
PYS (parietal yolk sac carcinoma)	129	Ascites	Intraperitoneum (20–30%)	Ascites

routinely for examination under UV to ensure the accuracy of the identification routinely made with phase microscopy.

Blastocysts chosen for the experiments were injected into the appropriate site in the animal (Table 1), and the appropriate number of cells were injected into the same anatomical site to serve as controls.

RESULTS

The data obtained with 402Aẍ are listed in Table 2. Single 402Aẍ cells injected into blastocysts have about one-half the chance of producing tumors as do controls. This observation is statistically significant at the 0.025 level, but the degree of change is not as marked as that reported previously (23). Although this difference cannot be explained with certainty, it probably reflects the improved technology (see "Materials and Methods") and/or repeated cloning which resulted in progression of the tumor.

402Aẍ was originally chosen for this assay because it cloned easily from the ascites, but this advantage was obviously outweighed by the degree of difference in tumorigenesis between experimental and control groups exhibited in the current experiments. Accordingly, 2 other lines of embryonal carcinoma were tested as potentially better candidates for the assay. Experiments with F-9 were extremely reproducible, but the single cells showed no reduction in tumorigenesis when injected into blastocysts (Table 2). The tumors that grew from single F-9 cells injected into blastocysts were indistinguishable from those grown from single cells without the blastocyst.

Embryonal carcinoma 247 was chosen for study because it was believed that a line of embryonal carcinoma that retained some capacity for differentiation into elements of the 3 germ layers might be more amenable to control by the blastocyst and therefore might be the best candidate for the assay. Although the line is extremely undifferentiated, it produced small foci of brain, and a few glands among large amounts of embryonal carcinoma when grown in the s.c. space. In an initial study, 15% of single cells (3 of 20) grown in the absence of blastocysts produced tumors, and 11% (3 of 27) produced tumors when injected into the perivitelline space. This indicated that we were not losing or injuring cells during the micromanipulations and that the homograft rejection of the blastocyst did not affect the growth of line 247 cells. The 11 to 15% cloning efficiency was thought to be too low, however, for efficient use in the assay.

Because others had produced chimeras from multiple embryonal carcinoma cells injected into the blastocysts (18, 19), we decided to use 5 cells in the tumorigenicity assay. When 5 cells were injected per testis, tumors were obtained in 11 of 35 cases or in 35%. This was considered to be a reasonable base line for the assay, although the problem of leakage of injected cells was greater than that observed when single cells were used and reached 50% of blastocysts which were given injections. All blastocysts were kept under observation to ensure that only those containing 5 cells in their cavities were used in the experiments. The data are listed in Table 2. When the incidence of tumors obtained with 5 cells in the blastocele was compared to that of 5 cells in the perivitelline space, it was clear that the blastocyst was efficient in reducing tumorigenicity of line 247 cells; observations were significant at the 0.025 level.

Specificity of the reduction of tumorigenicity by the blastocyst was tested indirectly by determining if the blastocyst could control Sarcoma 180 cells, L1210 leukemia cells, parietal yolk sac carcinoma cells, and C1300 neuroblastoma cells. Whereas Sarcoma 180, L1210 leukemia, and parietal yolk sac carcinomas were not affected by the blastocyst (no statistically significant difference), C1300 neuroblastoma consistently showed evidence of reduced tumorigenicity (significance level, 0.05). The data are shown in Table 3.

DISCUSSION

The purpose of these experiments was to use a variety of tumors to probe the specificity of the mechanism whereby the blastocyst regulates tumor formation of certain embryonal carcinoma cells.

When blastocysts containing single or small numbers of embryonal carcinoma cells are put into the uteri of foster mothers, 3 results have been reported in the newborn mice; a few are chimeras, a few develop tumors, and the rest are not chimeras and do not have tumors (10, 19). The production of chimeras is compatible with the idea that the blastocyst regulated or controlled the differentiation of the cancer cell in such a manner that the progeny could take part in normal development. The fate of the cancer cells in blastocysts that failed to produce either chimeras or tumors is not known (5, 10, 16, 18, 19).

In our assay, only the tumor-forming capacity of malignant cells is measured. In those situations in which tumors did not develop, it is possible that some of the embryonal carcinoma cells were induced to differentiate. Although this has not been demonstrated at the present time, the idea is compatible with the experience in the production of chimeric animals.

Because dead cells have been reported in blastocysts (8), it might also be postulated that the blastocyst has a mechanism of destroying aberrant cells. No support for such a possibility has been found in our experiments. B-16 melanoma (23), L1210, Sarcoma 180, and parietal yolk sac carcinoma cells

Table 2
Growth of tumor cells in vivo

Tumor	No. of Cells	No. of tumors/no. of animals given injections: In absence of blastocyst	In blastocysts
402Aẍ	1	37/93 (40)[a]	21/93 (23)[b]
F-9	1	49/147 (33)	43/123 (35)
247	5	15/41 (37)[c]	3/38 (8)[b]

[a] Numbers in parentheses, percentage.
[b] The reduction in tumorigenicity of cells in the blastocyst is statistically significant ($\alpha < 0.025$).
[c] In this series, 5 control cells were placed in the perivitelline space. See "Results" and "Discussion."

Table 3
Growth of single tumor cells

Tumor	No. of animals/no. of animals given injections: In absence of blastocyst	In blastocysts
Sarcoma 180	16/80 (20)[a]	16/70 (23)
L1210	29/85 (34)	20/70 (29)
PYS carcinoma	34/131 (26)	24/117 (21)
C1300	58/123 (47)	44/126 (35)[b]

[a] Numbers in parentheses, percentage.
[b] The reduction in tumorigenicity of cells exposed to the blastocyst is statistically significant ($\alpha < 0.05$). The effect of the blastocyst on all other tumors is insignificant statistically.

grow almost equally well when cloned within blastocysts or in the absence of blastocysts. Through the use of labeled cells and marker techniques with the electron microscope, we hope to obtain data upon the fate of embryonal carcinoma cells that fail to form tumors after injection into blastocysts.

Irrespective of the fate of the injected embryonal carcinoma cells, the data make it clear that the blastocyst reduces tumorigenicity for 2 of 3 lines of embryonal carcinomas tested. This is in accord with the data of Papaioannou *et al.* (18), who injected aggregates of the C145 line of embryonal carcinoma into blastocysts, which were injected beneath the renal capsule. Three of 18 developed tumors in comparison to 20 of 20 animals that received aggregates without blastocysts. In addition, our data are compatible with the idea that the reduction in tumorigenicity may be specific for embryonal carcinoma.

Gardner, who first developed the techniques for production of "injection" chimeras (8), and later Moustafa and Brinster (17) showed that there must be relatively close correspondence in the developmental age of the blastocyst and the injected normal cell for the production of a chimeric mouse. Embryonal carcinoma is believed to be the malignant counterpart of the inner cell mass of the blastula; therefore, its regulation by the blastocyst is in keeping with the idea that there must be a close correspondence between embryonic field and injected cell for regulation to occur.

To examine the premise that reduction in tumor formation might be restricted to tumors with close counterparts in the embryonic field, 3 tumors that lacked a counterpart in the blastula and one with such a counterpart were chosen for study. The apparent lack of control of B16 melanoma cells by the blastocyst reported previously (25) was extended by the observation that tumor-forming capacity of neither L1210 nor Sarcoma 180 was controlled by the blastocyst. What might be interpreted as discordant observations with theory were made with the C1300 neuroblastoma and the parietal yolk sac carcinoma.

It is clear from the data that C1300 cells were less tumorigenic when single cells were injected into the blastocele than when controls were cloned in the absence of blastocysts. Since inner cell mass cells differentiate differently into primitive neural tissue of the embryo 3 to 4 days after blastulation (32), it is conceivable that the cancer cells persisted in the embryo and that tumor formation was abrogated at the time of neurulation. Although the data are significant only at the 5% level, we have commenced a series of experiments using the techniques of Beddington (2) for culture of postimplantation embryos to determine if the neurula can abrogate tumor formation of neuroblastoma cells.

We have no explanation for the lack of effect of the blastocyst upon the parietal yolk sac carcinoma. Because normal endoderm differentiates from the inner cell mass of the late blastula, it was anticipated that the parietal yolk sac carcinoma would be regulated by the blastocyst. Because only certain lines of embryonal carcinoma can be regulated by the blastocyst, it is conceivable that only certain lines of parietal yolk sac carcinoma will be responsive. Other lines of this tumor must be assayed before final conclusions with regard to parietal yolk sac carcinomas can be drawn. It must be remembered that the extraembryonic differentiations of embryonal carcinoma (choriocarcinoma and yolk sac carcinoma) are exceptions to the general rule that tissues derived from embryonal carcinoma are benign (20). Possibly, tumors of extraembryonic fetal membranes are not amenable to embryonic control.

Whatever the mechanism of these experiments, it is clear that some embryonal carcinoma cells respond negatively to an embryonic environment in terms of tumor formation, but we have also observed what can be interpreted as positive control or enhancement of malignancy by another cellular environment related to the early embryo (24). Neither 402Ax̄ nor the parietal yolk sac carcinomas spontaneously metastasize from the ascites. If a single cell from either tumor is incubated on a monolayer of embryonic cells for 24 hr and the preparations (including glass substrate, monolayer, and cancer cell) are transferred to the peritoneal cavity, ascites forms of the tumor develop, and gross metastases to lymph nodes and lungs occur in high frequency (24). It is not known if the cancer cells are induced to metastasize by the embryonic cells or by the fact that they are anchored to a substrate. In support of the latter ideas is the observation that tumor formation by 3T3 cells could be demonstrated only if the cells were attached to a glass bead (4).

Just as the environment is important in phenotypic expression in developing systems, it appears to be equally important in neoplastic ones. Detailed studies of the immediate cellular environments of tumors must be made if we are to understand how malignant cells are regulated. In this regard, use of chemically defined media will be useful (26).

The dream of developing an alternative to cytotoxic therapy for cancer by directing differentiation of malignant to benign cells through the use of naturally occurring specific embryonic inductors is still a dream. On the basis of experiments reported here, abrogation of tumorigenicity of embryonal carcinoma cells by the blastocyst appears to be specific in terms of correspondence of the neoplastic cells and embryonic field. We believe that, if one embryonic environment can control some of its closely related cancers, then there may be an embryonic environment appropriate for control of each type of cancer.

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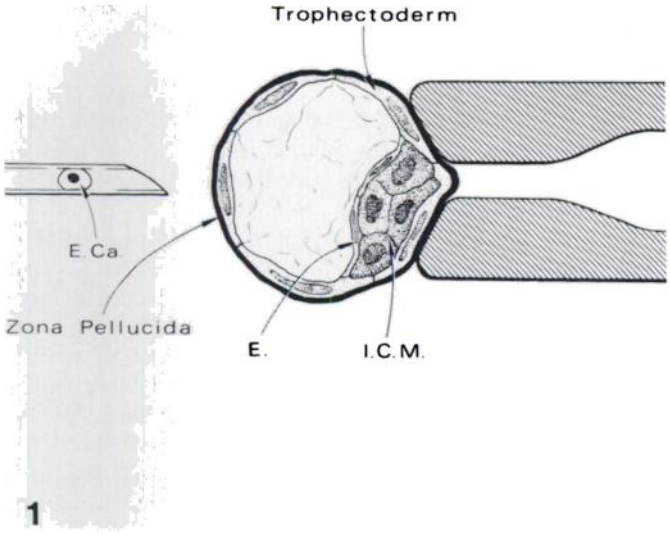
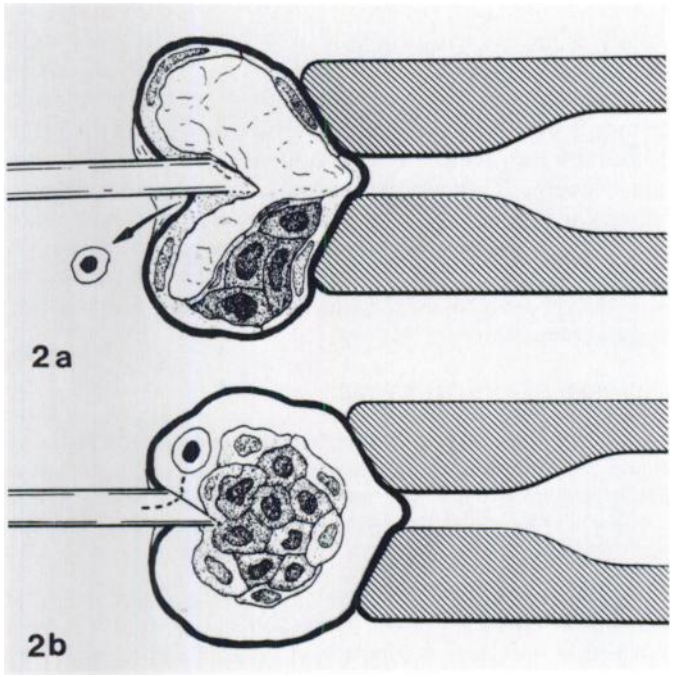
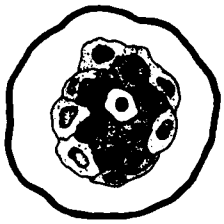
REFERENCES

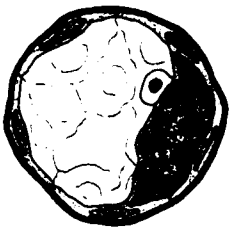
1. Babinet, C. A simplified method for mouse blastocyst injection. Exp. Cell Res., *130:* 15–19, 1980.
2. Beddington, R. S. P. An autoradiographic analysis of the potency of embryonic ectoderm in the 8th day post implantation embryo. J. Embryol. Exp. Morphol., *64:* 87–104, 1981.
3. Bernstine, E. G., Hooper, M. L., Grandchamp, S., and Ephrussi, B. Alkaline phosphatase activity in mouse teratoma. Proc. Natl. Acad. Sci. U. S. A., *70:* 3899–3903, 1973.
4. Boone, C. W.: Malignant hemangioendotheliomas produced by subcutaneous inoculation of Balb/3T3 cells attached to glass beads. Science (Wash. D.C.), *188:* 68–79, 1975.
5. Brinster, R. L. The effect of cells transferred into the mouse blastocyst on subsequent development. J. Exp. Med., *140:* 1049–1056, 1974.
6. de Lustig, E. S. and Matos, E. L. Regeneration and carcinogenesis: carcinogenesis in regenerating tadpoles tails of *Bufo arenarum. In:* R. Harris, P. Allin, and D. Viza (eds.), Cell Differentiation, pp. 124–130. Copenhagen: Einer Munksgaard, 1972.
7. Friend, C., Preisler, H. D., and Scher, W. Studies on the control of differentiation of murine virus-induced erythroleukemia cells. Curr. Top. Dev. Biol., *8:* 81–101, 1974.
8. Gardner, R. L. Mouse chimaeras obtained by the injection of cells into the blastocyst. Nature (Lond.), *220:* 596–597, 1968.
9. Glucksman, A., and Cherry, C. P. Incidence, histology and response to

radiation of mixed carcinomas (adenoacanthomas) of the exterior cervix. Cancer (Phila.), *9:* 971–979, 1956.
10. Illmensee, K. Reversion of malignancy and normalized differentiation of teratocarcinoma cells in chimeric mice. *In:* L. Russel (ed.), Genetic Mosaics and Chimeras in Mammals, pp. 3–25. New York: Plenum Publishing Corp., 1978.
11. Isa, A. M., and Sanders, B. R. Lack of demonstrable H-2 antigens on the surface of teratocarcinoma 402Aẍ of the mouse maintained *in vivo*. Transplantation (Baltimore), *21:* 195–198, 1976.
12. Kleinsmith, L. J., and Pierce, G. B. Multipotentiality of single embryonal carcinoma cells. Cancer Res., *24:* 1544–1551, 1964.
13. Lehman, J., Speers, W. C., Swartzendruber, D. E., and Pierce, G. B. Neoplastic differentiation: characteristics of cell lines derived from a murine teratocarcinoma. J. Cell Physiol., *84:* 13–28, 1974.
14. Mantel, N., and Haenszel, W. Statistical aspects of the analysis of data from retrospective studies of disease. J. Natl. Cancer Inst., *22:* 719–748, 1959.
15. Markert, C. L., and Petters, R. M. Homozygous mouse embryos produced by microsurgery. J. Exp. Zool., *201:* 295–302, 1977.
16. Mintz, B., and Illmensee, K. Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc. Natl. Acad. Sci. U. S. A., *72:* 3585–3589, 1975.
17. Moustafa, L. A., and Brinster, R. L. Induced chimaerism by transplanting embryonic cells into mouse blastocysts. J. Exp. Zool., *181:* 193–201, 1972.
18. Papaioannou, V. E., McBurney, M. W., Gardner, R. L., and Evans, R. L. Fate of teratocarcinoma cells injected into early mouse embryos. Nature (Lond.), *258* :70–73, 1975.
19. Papaioannou, V. E., Evans, E. P. Gardner, R. L., and Graham, C. F. Growth and differentiation of an embryonal carcinoma cell line (C145b). J. Embryol. Exp. Morphol., *54:* 277–295, 1979.
20. Pierce, G. B. Teratocarcinoma: model for a developmental concept of cancer. Curr. Top. Dev. Biol., *2:* 223–246, 1967.
21. Pierce, G. B., and Dixon, F. J. Testicular teratomas. I. The demonstration of teratogenesis by metamorphosis of multipotential cells. Cancer (Phila.), *12:* 573–583, 1959.
22. Pierce, G. B., Dixon, F. J., and Verney, E. L. Teratocarcinogenic and tissue forming potentials of the cell types comprising neoplastic embryoid bodies. Lab. Invest., *9:* 583–602, 1960.
23. Pierce, G. B., Lewis, S. H., Miller, G. J., Moritz, E., and Miller, P. Tumorigenicity of embryonal carcinoma as an assay to study control of malignancy by the murine blastocyst. Proc. Natl. Acad. Sci. U. S. A., *76:* 6649–6651, 1979.
24. Pierce, G. B., Pantazis, C. G., Caldwell, J. E., and Wells, R. S. Embryologic control of malignancy. *In:* C. Harris and P. Ceruth (eds.), Mechanisms of Chemical Carcinogenesis, J. Supramol. Struct. Cell Biochem., 1982.
25. Pierce, G. B., and Verney, E. L. An *in vitro* and *in vivo* study of differentiation in teratocarcinomas. Cancer (Phila.), *14:* 1017–1029, 1961.
26. Rizzino, A., and Sato, G. Growth of embryonal carcinoma in serum free medium. Proc. Natl. Acad. Sci. U. S. A., *75:* 1844–1848, 1978.
27. Seilern-Aspang, F., and Kratochwil, K. Die Unduktion von knorpelbildung in einem Hühnersarkom *in vivo* durch Implantation embryonaler Organe des Hühnchens. Acta Biol. Med. Ger., *10:* 443–446, 1963.
28. Seilern-Aspang, F., and Kratochwil, K. Induction and differentiation of an epithelial tumor in the newt (*Triturus cristatus*). J. Embryol. Exp. Morphol., *10:* 337–356, 1962.
29. Seilern-Aspang, F., and Weissberg, M. Die einwirkung verschieden alter embryonaler homogenate aud ein chemisch induzierles Sarkom des Hühnes. Acta Biol. Med. Ger., *10:* 439–442, 1963.
30. Sherman, M. I. The culture of cells derived from mouse blastocysts. Cell, *5:* 343–349, 1975.
31. Silagi, S., and Bruce, S. A. Suppression of malignancy and differentiation in melanotic melanoma cells. Proc. Natl. Acad. Sci. U. S. A., *66:* 72–78, 1970.
32. Snell, S. D., and Stevens, L. C. Early embryology. *In:* E. L. Grun (ed.), The Biology of the Laboratory Mouse, Ed. 2, pp. 205–245. New York: Dover Publication, 1968.
33. Strickland, S., and Sawey, M. J. Studies on the effect of retinoids on the differentiation of teratocarcinoma stem cells *in vitro* and *in vivo*. Dev. Biol., *78:* 76–85, 1980.
34. Whisson, M. E. The interaction of tumor and embryonic tissues *in vivo*. *In:* A. V. S. De Reuck and J. Knight (eds.), Cell Differentiation, CIBA Foundation Symposium, pp. 219–230. London: J & A Churchill, 1967.

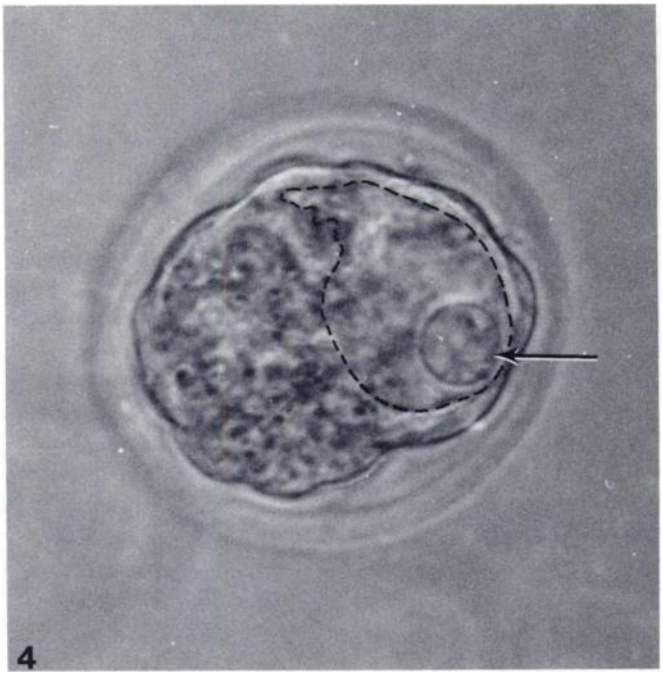








3 Collapsed Expanded



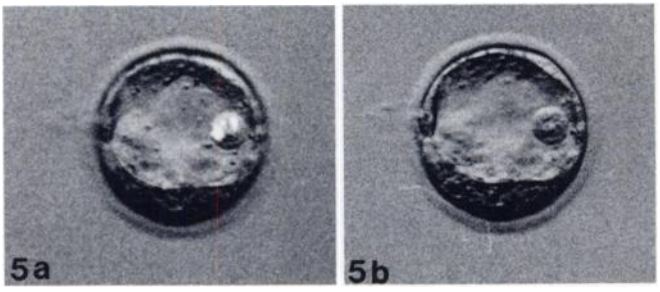


Fig. 1. Diagram of the techniques used. *Right*, holding pipet. Blastocyst is attached to it by suction. This is a late blastocyst which is about 80 μm in diameter and is surrounded by the zona pellucida. It is composed of a layer of trophectoderm which synthesizes the blastocele fluid. The inner cell mass (*I.C.M.*) will form the cells of the embryo proper, and a thin layer of endoderm lies on its surface (*E*). The injecting pipet holds an embryonal carcinoma cell (*E.Ca.*).

Fig. 2. In *a*, if the injecting pipet does not penetrate the trophectoderm to enter the blastocele, the injected cell either escapes from the blastocyst or becomes entrapped between the zona pellucida and the trophectoderm (perivitelline space). Embryonal carcinoma cells in the perivitelline space are not controlled. In *b*, if the blastocele collapses, the cell can be inadvertently placed in the perivitelline space.

Fig. 3. After injection, the blastocyst often collapses into a ball of cells; the cancer cell cannot be seen in it. When the trophectoderm resynthesizes the blastocele fluid, the cancer cell is easily seen in the expanded blastocele.

Fig. 4. Photomicrograph of partially reexpanded blastocyst containing a cancer cell (*arrow*). The blastocele has been outlined. × 650.

Fig. 5. Fully expanded blastocyst containing a single 247 cell labeled with polystyrene microspheres. *a*, phase and fluorescence microscopy; *b*, phase microscopy. Both × 100.