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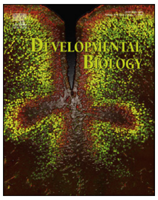

Commentary

# Commentary on “Direct visualization, by β-galactosidase histochemistry, of differentiated normal cells derived from malignant teratocarcinoma in allophenic mice” by Dewey and Mintz 1978

To put this publication into historical context, embryonic stem cells had not yet been derived, but a number of investigators in the British and US mouse embryology groups were thinking about the possibility of developing stem cells that could contribute to the embryo. Teratomas and teratocarcinomas, which occasionally arose in domestic animals, had long fascinated biologists because the multiple embryonic cell and tissue types represented in these tumors implied that they derived from an embryonic stem cell present in the early embryo. Once Leroy Stevens discovered several mouse strains with a high incidence of teratocarcinomas, they became the first tractable approach for deriving stem cells that might contribute to the embryo.

The Mintz lab had developed teratocarcinoma cell lines from 129 XY and LT XX mice, and, by the mid-nineteen seventies, shown that they could be propagated in vitro, cryopreserved, and selected for loss of *Hprt* – to my knowledge, the first intentional genetic modification induced in a stem cell line with the goal of introducing a genetic change into an embryo. In a 1977 paper, Dewey and MIntz had shown that these modified teratocarcinoma cells could be introduced into a blastocyst of a different inbred strain, where they contributed to multiple tissues in the “allophenic” embryo. To estimate contribution of the teratocarcinoma cells to various embryonic tissues, the tissue was homogenized, and hemoglobin or immunoglobulin types, or enzyme variants characteristic of the two strains were analyzed. These assays confirmed the existence of mosaicism for strain specific variants, and implied that the original malignant teratocarcinoma cells had differentiated to produce cell-type specific products. However questions remained about whether the cells (1) had integrated into the tissue and adopted typical morphology, and (2) had not produced tumors in the mouse.

This 1978 paper took advantage of an *in situ* technique developed earlier by the lab to examine contribution of donor strains in chimeras produced from aggregation of early embryos. Dewey showed that levels of β-galactocidase detected by a histochemical assay *in situ* differed between the contributing strains in different tissues. In this paper, he showed that pockets of differentiated cells derived from the teratocarcinoma existed in multiple tissues where they had differentiated normally. Although not all tissue types could be assayed using this approach, this paper showed that teratocarcinoma cells convincingly contributed to the exocrine pancreas and Purkinje cells. All tissues arising from the teratocarcinoma cells were indistinguishable in morphology and differentiation from adult control cells, and showed no malignant growth.

Of course, ultimately, malignant teratocarcinoma cells did not become the embryonic stem cell of choice. However, the ideas behind this work, and the evidence that a cultured stem cell – even one that had been genetically modified – could contribute cells to many tissues in the embryo that adopted normal differentiation patterns in the context of the embryo, opened a vista onto what might be possible, and set the stage for the rapid advances that followed.

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# Direct Visualization, by β-Galactosidase Histochemistry, of Differentiated Normal Cells Derived from Malignant Teratocarcinoma in Allophenic Mice

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Mice obtained from blastocysts injected with malignant teratocarcinoma stem cells may comprise tumor-derived cells in their tissues. Evidence for their presence has hitherto been indirect, i.e., through detection of tissue-specific products of the tumor genotype or of strain-specific enzyme variants in tissue homogenates from healthy mice. Direct visualization and identification of the tumor-derived cells would permit their normalcy and their state of differentiation to be assessed. For this purpose, a histochemical marker is required. The marker chosen was β-galactosidase (BGS), which allows high- vs low-activity cell strains to be distinguished *in situ* by their differences in staining intensity. BGS has previously been employed for such visualization only in brain [Dewey, M., Gervais, A., and Mintz, B. (1976). *Develop. Biol.* **50**, 68–81] and has here been shown to be applicable to other tissues, including kidney, pancreas, and salivary gland. Two unexpected results concerning the marker itself were obtained and affected its application to histochemical comparisons: BGS activity in some tissues of some inbred strains was not concordant with that of brain, on which the existing genotypic classification is based; and some cell types within a tissue varied independently in BGS levels among strains (e.g., exocrine vs endocrine pancreas). BGS visualization clearly disclosed the presence of large numbers of *fully differentiated normal cells of the teratocarcinoma strain* in tissues, including the Purkinje layer of the cerebellum, the kidney tubules, and the exocrine pancreas of experimental animals. In one individual, the relevant brain region was almost entirely derived from the teratocarcinoma. Yet all tissues were indistinguishable in structure and differentiation from adult controls, and showed no malignant growth. The pattern of cell-strain distribution, which was fine-grained in the brain and patchy in the other tissues named, also resembled that of ordinary allophenic mice produced from blastomere aggregates of two strains. Thus, teratocarcinoma stem cells are here seen to undergo normal histogenesis after they are successfully incorporated into a developing host embryo.

## INTRODUCTION

Malignant mouse teratocarcinoma stem cells, after introduction into normal embryos at the blastocyst stage, have been found to participate in embryogenesis along with blastocyst-derived cells (Mintz *et al.*, 1975; Mintz and Illmensee, 1975; Illmensee and Mintz, 1976). Mutagenized teratocarcinoma stem cells, selected in culture for hypoxanthine phosphoribosyltransferase deficiency—the same genetic lesion as occurs in human Lesch–Nyhan disease—have also proven to be developmentally totipotent after injection into blastocysts (Dewey *et al.*, 1977). The differentiated and functional status of the tumor-derived cells has been documented in numerous tissues of these allophenic mice by means of tissue-specific products (e.g., melanins, hemoglobin, immunoglobulins) of the tumor strain, and by the occurrence, in tissue homogenates, of strain-specific electrophoretic variants of widely distributed enzymes (e.g., glucose phosphate isomerase). The present study was undertaken in order to obtain more direct evidence of the fully differentiated and nonmalignant state of the tumor-lineage cells.

This aim can best be accomplished by visualizing the two cellular genotypes his-

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tochemically in tissue sections. Only two markers have been utilized in allophenic mice for genotypic identification *in situ*: β-glucuronidase (Condamine *et al.,* 1971; Feder, 1976) and β-galactosidase (Dewey *et al.,* 1976); they permit cell strains to be distinguished by virtue of strain differences in enzyme activity. Both markers have thus far been applied only to mice produced from aggregated blastomeres of two strains (Mintz, 1974). β-Galactosidase (BGS) was chosen here for analyses of teratocarcinoma-lineage allophenic mice because of its applicability to the particular strain combinations involved. This enzyme has previously been examined only in brains of mosaic animals (Dewey *et al.,* 1976). We report here that it is also a good strain discriminant in histochemical preparations of various other tissues. In addition, as described in more detail in the following paper (Dewey and Mintz, 1978), it was found unexpectedly to have interesting complexities of gene control.

BGS visualization of the tumor-strain contribution in tissues from the experimental animals clearly demonstrates that the cells are all normal and fully differentiated and are indistinguishable from controls. Moreover, the pattern of distribution of the two cell strains within mosaic tissues indicates that both strains have jointly participated in normal histogenesis.

## MATERIALS AND METHODS

*Mice.* The allophenic mice used in this study (Table 2) were obtained from blastocysts injected with malignant teratocarcinoma stem cells. One mouse [case No. 1, here and in Mintz and Illmensee (1975)] developed from a C57BL/6 (*brown, b/b*) embryo injected with cells of the OTT 6050 tumor transplant line of strain 129/Sv $Sl^J$ $C$ $P$ (129). The tumor-lineage cells have been found to be chromosomally male (X/Y) and euploid; the blastocyst-derived cells were female (X/X) (Cronmiller and Mintz, 1978). The other mouse (No. 2) was from a blastocyst of the CBA strain (with the homozygous T6/T6 translocation) injected with stem cells of a transplanted ovarian teratocarcinoma (72484-395) of the LT strain (Mintz and Illmensee, manuscript in preparation). These tumor cells were euploid and X/X and the blastocyst was X/X (Cronmiller and Mintz, 1978). Both animals contained significant contributions of tumor-derived normal cells in the coat, germ line, and other tissues (e.g., blood) tested while they were alive.

At autopsy, part of each major internal tissue was homogenized and the strain genotypes were estimated from the proportion of strain-specific electrophoretic variants of glucose phosphate isomerase or isocitrate dehydrogenase, by starch gel electrophoretic methods (Gearhart and Mintz, 1972; Mintz and Baker, 1967). In Table 2, the isozyme results are presented for those tissues to which the BGS histochemical marker was also applied. Further details of tissue composition, from isozyme analyses of homogenates, will be given elsewhere (Mintz and Illmensee, manuscript in preparation). In addition to tests for electrophoretic variants, cryostat sections (with controls) were prepared, for BGS visualization, from those tissues first found in control studies to be favorable for histochemical diagnoses of genotypes.

*β-Galactosidase histochemical and biochemical determinations.* Histochemical visualization of BGS was performed according to the method of Lojda (1973), with the substrate 4-chloro-5-bromo-3-indolyl-β-D-galactoside. The procedure separates the tissue section from the substrate by a dialysis membrane which prevents extensive diffusion of the enzyme. Allophenic tissues and the corresponding pure strains were snap-frozen together on the same chuck, as described previously (Dewey *et al.,* 1976), to control for minor variations in section thickness. Scattered sample cryostat sections 10 or 15 μm thick were cut from each tissue.

Levels of BGS activity in tissue homogenates were determined with the substrate *p*-nitrophenyl-β-D-galactoside, as described by Felton *et al.* (1974), except that the homogenates were prepared in a Ten Broeck tissue grinder and subjected to one freeze–thaw cycle prior to assay.

## RESULTS

### *Relative BGS Activities in Control Tissue Homogenates*

The specific activity of BGS was first determined biochemically in homogenates of brain, kidney, liver, and salivary (submaxillary) gland, for the 129, C57BL/6, CBA, and LT strains composing the allophenic animals used in this study. The results were then expressed as *relative activities*: a rating of "high" indicates that the level was 1.7 or more times higher than that of the strain (among the four tested) having the lowest activity in that tissue (Table 1). In the case of these four tissues, the histochemical results paralleled the biochemical assays. This was not true for pancreas, however. In that case, the exocrine and endocrine BGS levels were found to be independently regulated among the strains (Dewey and Mintz, 1978). Therefore, for histochemical purposes in the present study, the values for exocrine pancreas given in Table 1 are based on the relative histochemical staining intensities (Fig. 2) rather than on the biochemical assays of whole pancreas homogenates.

In previous work, *Bgs*-locus genotypes (i.e., $Bgs^h$ for high activity, or $Bgs^d$ for diminished activity) were assigned solely on the basis of the level of BGS activity in brain (Felton *et al.*, 1974). From the present results (Table 1) and further details in Dewey and Mintz (1978), it is apparent that other tissues of the same strains do not show concordance with the brain data. Examples are seen in the salivary glands of the three tested strains and in the pancreas of C57BL/6. Thus, some tissues might lend themselves to histochemical application of the BGS marker in allophenic strain combinations where the marker cannot be used in brain, and vice versa. In other tissues of appropriate strain pairs, the identity of high and low strains would be the reverse of that in brain.

### *BGS Marker Evaluation in Histochemical Preparations of Control Tissues*

The BGS histochemical evidence in all tissues showed differences in *overall* staining intensity commensurate with their biochemically ascertained relative activities (Table 1). However, the various cell types comprising a tissue displayed marked disparities in enzyme activity (see controls, Figs. 1–4). A similar observation was made when the enzyme glucose phosphate isomerase was visualized histochemically (Dewey *et al.*, 1976).

TABLE 1

RELATIVE ACTIVITIES OF β-GALACTOSIDASE (BGS) IN TISSUES OF CONTROL MOUSE STRAINS

| Inbred strain | *Bgs* allele[a] | Relative BGS activities[b] | | | | |
|---|---|---|---|---|---|---|
| | | Brain | Kidney | Liver | Salivary gland[c] | Exocrine pancreas |
| 129 | *d* | Low | Low | Low | High | Low |
| C57BL/6 | *h* | High | High | High | Low | Low |
| CBA | *d* | Low | Low | Low | (N.T.) | Low |
| LT | *h* | High | High | High | High | High |

[a] The *Bgs* genotypes for strains 129, C57BL/6, and CBA, based on brain, are taken from Felton *et al.* (1974). The allele for LT (not hitherto published) was determined to be $Bgs^h$, also from the BGS activity in brain.

[b] For brain, kidney, liver, and salivary gland, a relative score of "high" was assigned if the specific activity of BGS in tissue homogenates was at least 1.7 times higher than that of the strain (from the four listed) with the lowest activity in that tissue. For exocrine pancreas, the relative values were assigned from comparisons of the histochemical staining intensities [see text and Dewey and Mintz (1978) for details].

[c] Submaxillary gland.

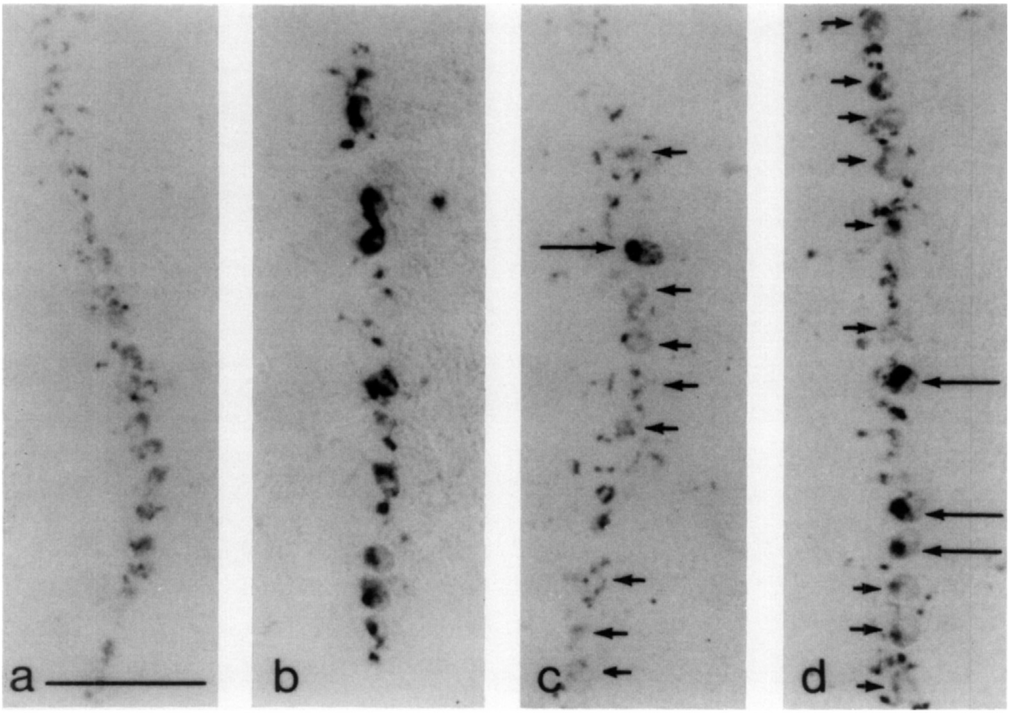

FIG. 1. Cryostat sections of cerebellum stained for BGS activity. (a) A 129-strain control, with low activity (*Bgs*$^d$); (b) C57BL/6-strain control, with high activity (*Bgs*$^h$); (c) C57BL/6 ↔ 129 allophenic male mouse No. 1, obtained from a C57BL/6$^{(b/b)}$ blastocyst injected with 129-strain malignant teratocarcinoma cells; (d) CBA ↔ LT allophenic female mouse No. 2 from a CBA (T6/T6) blastocyst injected with LT-strain teratocarcinoma cells. (The CBA control brain is low-activity, like 129, and the LT is high-activity, like C57BL/6.) The two controls show a clear difference in staining of Purkinje cells. In the mosaic brain of mouse No. 1 (c), the difference allows identification of a *single* blastocyst-derived Purkinje cell (long arrow) in a string of *all-teratocarcinoma-descended cells* (short arrows). This brain has both chromosomally male (X/Y) cells, from the teratocarcinoma, and chromosomally female (X/X) cells, from the blastocyst. Teratocarcinoma-derived Purkinje cells in mouse No. 2 (d) are high-activity and are shown with long arrows; blastocyst-derived cells are shown with short arrows. (Bergmann glia are seen as stained punctate cells.) Bar, 100 μm.

In the exocrine pancreas, strain differences were especially dramatic, with fairly uniform BGS staining throughout the acini (Figs. 2a and b). The BGS histochemical marker is clearly favorable to distinguish *in situ* the two genotypes in mosaic pancreas.

As previously reported (Dewey *et al.,* 1976) and demonstrated here (Figs. 1a and b), the predominant BGS-containing cell type of the cerebellum is the Purkinje cell. There is some variation in the staining intensity from cell to cell of a given strain, probably as a result of the inclusion of variable portions of the lysosome-containing region in the section. Nearby Bergmann glia cells are also stained.

BGS activity is marked in the cortex of kidney (Figs. 3a and b) and low in the medulla. The cortical activity is primarily in the convoluted tubules, although a great deal of variation in intensity is seen within the same section. This variability, which may be due in part to regional differences within a tubule, complicates the application of the BGS marker to studies of allophenic kidney and suggests a need for serial sections for a full genotypic evaluation. Nevertheless, in kidney sections of the low genotype, the most highly stained tubules are never as intense as the most reactive in the high strain. Thus, the marker should be used primarily to determine whether there

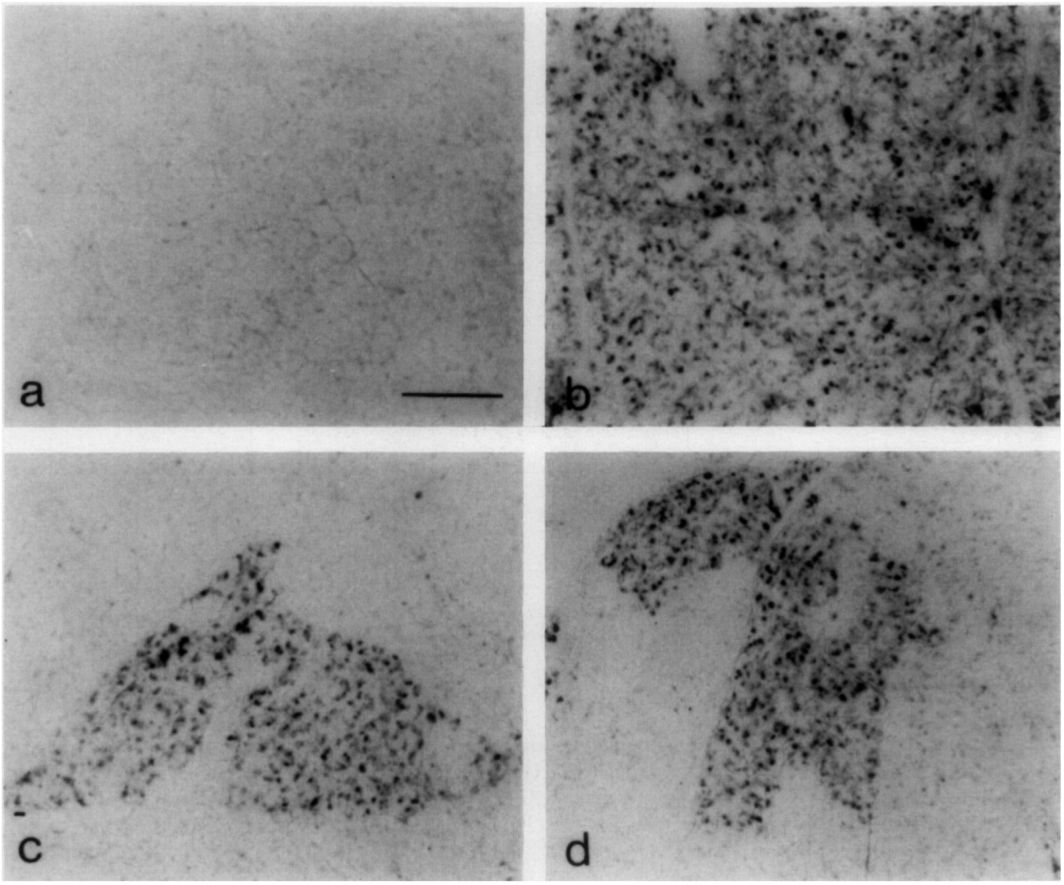

FIG. 2. Pancreas sections stained for BGS activity. (a) CBA (low-activity-strain control); (b) LT (high-activity-strain control); (c, d) CBA ↔ LT allophenic female mouse No. 2, from a CBA blastocyst injected with LT-strain teratocarcinoma cells. The staining reaction of the exocrine pancreas (acini) is strikingly different in the pure-strain controls. In the experimental, mosaicism is evident from the large clearly defined patches of high-reactivity acini surrounded by low-staining cells. Bar, 100 μm.

is a high-strain component in a kidney that is predominantly low-strain.

BGS histochemical preparations of liver reveal numerous densely stained immigrant Kupffer cells, whose lysosomes are conspicuous with visualization of this lysosomal enzyme. The relatively low reactivity of parenchymal hepatocytes indicates that, unlike β-glucuronidase (Condamine *et al.*, 1971), BGS is not a good marker for histogenetic and clonal information about the hepatocytes.

Salivary gland (of which only submaxillary samples were screened) is another favorable tissue for histochemical application of the BGS marker. The collecting tubules exhibit good reactivity and stain fairly uniformly in the high-activity strain (Fig. 4). There is little activity in the acini.

It should be emphasized that not all tissues have been screened. Therefore, additional tissues in which the BGS histochemical marker could be employed may well be found.

### *In Situ Genotypic Analyses of Tissues in Allophenic Mice from Teratocarcinoma-Injected Blastocysts*

In none of the histochemically examined tissues from the experimental animals was

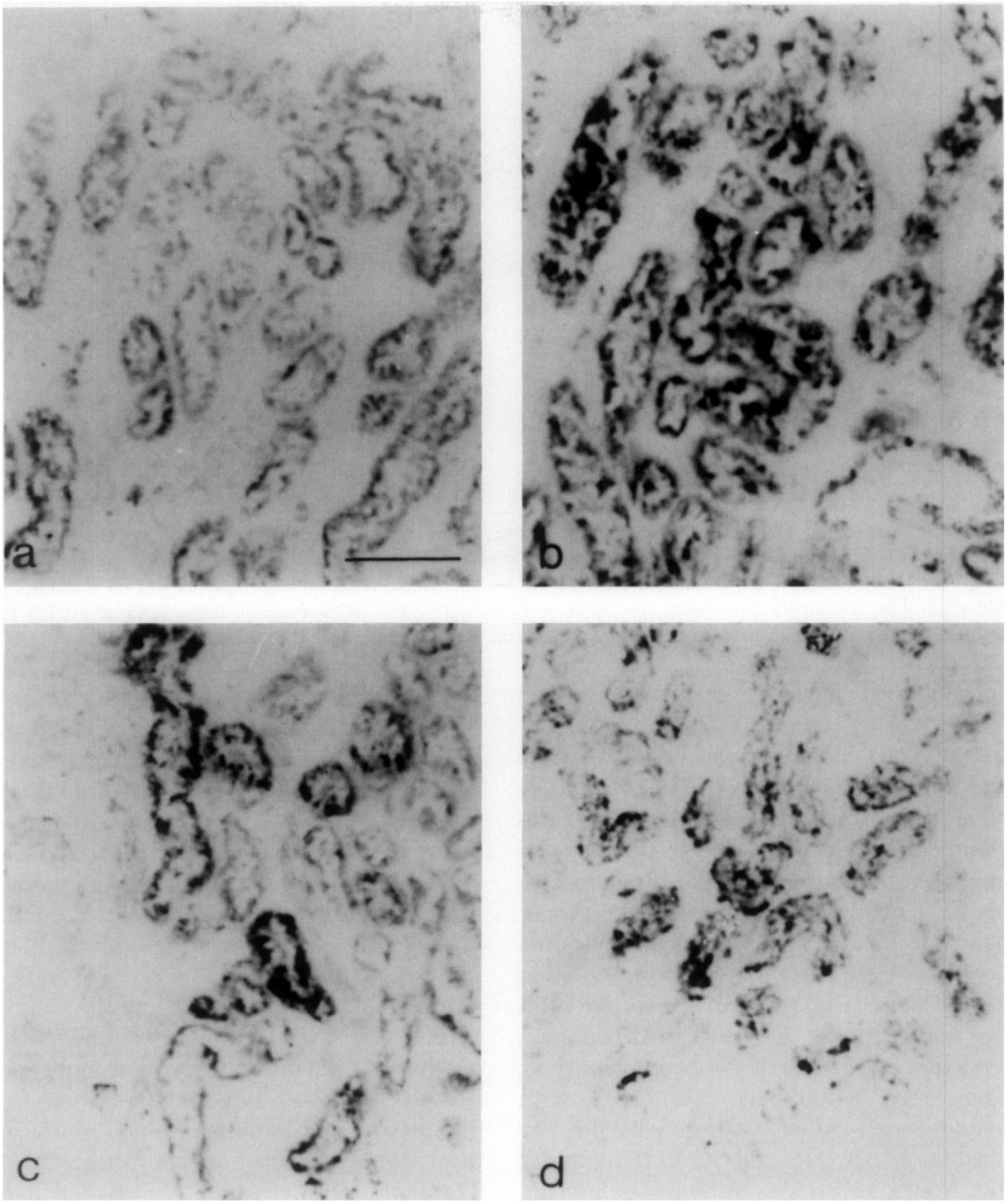

FIG. 3. Sections of kidney cortex stained for BGS activity. (a) CBA (low-activity-strain control); (b) LT (high-activity-strain control); (c) CBA ↔ LT allophenic female mouse No. 2; (d) C57BL/6 ↔ 129 allophenic male mouse No. 1. (The C57BL/6 control kidney is high-activity, like LT, and the 129 control is low-activity, like CBA.) The staining reaction is localized primarily to the convoluted tubules. The high- and low-strain controls reveal differences in reaction, with some variability within a given strain. Mosaicism in the allophenic kidneys is apparent from the clusters of high-strain tubules surrounded by others of the low strain. Bar, 100 $\mu$m.

there any indication of malignant growth, despite the presence of tumor-strain cells along with cells of the blastocyst strain. This was confirmed in hematoxylin-and-eosin-stained paraffin sections prepared for conventional histology from these tissues and others taken from the same animals.

The cerebellum of both animals clearly

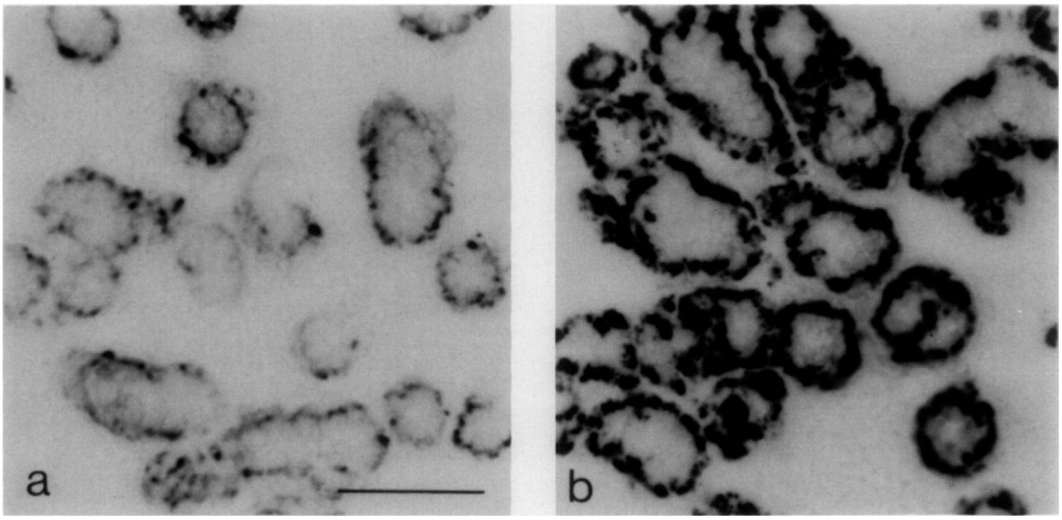

FIG. 4. Sections of submaxillary salivary gland from control males of the (a) low-activity C57BL/6 and (b) high-activity 129 strains. Note that the relative activities are the reverse of those in brain (Fig. 1) and kidney (Fig. 3) in these strains. The staining reaction is localized to the collecting tubules, with little or no reactivity in the acini. The uniformity of staining among the cells indicates that BGS is a good histochemical marker for cellular genotypic identification in mosaic salivary gland. Bar, 100 μm.

showed both genotypes of Purkinje cells in BGS-stained sections (Figs. 1c and d). The tumor-strain contribution in the two mice was substantially greater than that of the blastocyst strain, according to the histochemical evidence as well as the isozyme analyses of homogenates of cerebellum (Table 2). However, the tumor-strain component specifically localized in the Purkinje layer was even more heavily represented in mouse No. 1 (Fig. 1c) than was apparent in the homogenates. His cells in that region were *almost entirely* of the tumor genotype. They were, fortuitously, male (X/Y) with only a small minority (derived from the blastocyst) being female (X/X) (Cronmiller and Mintz, 1978). The animal was a fertile normal male and all progeny were from his X/Y germ cells of the tumor lineage (Mintz and Illmensee, 1975; further unpublished data).

The two genotypic classes of Purkinje cells are interspersed in a fine-grained distribution in each of the experimental mice. Single cells or small clusters of cells of one genotype, flanked by cells of the other, were commonly seen. An extreme example is shown in Fig. 1c: Here a *single* blastocyst-derived Purkinje cell (darkly stained) is seen in a long succession of tumor-derived Purkinje cells (lightly stained). This finely interdigitated pattern in the Purkinje layer is indistinguishable from the pattern observed (Dewey *et al.,* 1976) in the cerebellum of allophenic mice produced from all-normal cells, by aggregating embryo blastomeres of high- and low-activity paired strain combinations.

In the other tissues examined, the patches of cells of the respective genotypes were substantially larger, as is the case in many tissues of allophenic mice produced from aggregated blastomeres (Mintz, 1974). The most striking example observed here was in the exocrine pancreas. In mouse No. 2 (Figs. 2c and d), in which a minority population of tumor-derived (high-activity) acini was present, the cells of one genotype occur in large coherent groups that are frequently bordered and separated from patches of the other genotype by connective tissue septa.

In kidney, the variation in reaction intensity of convoluted tubules of the same strain

TABLE 2

Tumor-Strain Tissue Contributions[a] in Allophenic Mice Produced from Teratocarcinoma-Injected Blastocysts

| Mouse No. | Blastocyst strain | Teratocarcinoma strain | Phenotypic sex | Age (weeks) | Percentage teratocarcinoma-strain cells in | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Cerebellum | Pancreas | Kidney | Liver |
| 1 | C57BL/6 | 129 | ♂ | 89 | 75 | 85 | 70 | 85 |
| 2 | CBA | LT | ♀ | 61 | 75 | 15 | 10[b] | 5[b] |

[a] Estimated visually from the relative staining intensities of strain-specific electrophoretic enzyme variants in tissue homogenates. For comparison with histochemical diagnoses, based on visualization of BGS activity differences, see text and Figs. 1–3.

[b] The marker enzyme was isocitrate dehydrogenase in these cases and glucose phosphate isomerase in the remaining cases.

allowed only the most extremely high-staining of the high-genotype areas to be unequivocally identified in sample sections of mosaic tissue. As shown in Figs. 3c and d, sections of tubules with a large majority of high-activity cells can be discerned alongside tubules which also show significant, but reduced, contributions from the same strain. The distribution of cells of the two genotypes in convoluted tubules suggests an ordered arrangement in which the patch area extends in size beyond a single tubule. Similar observations have been made with the $\beta$-glucuronidase histochemical marker (Mintz, unpublished data) in allophenic mice comprising appropriate strain combinations of aggregated blastomeres.

## DISCUSSION

The absence of malignant growths in microscopically examined tissues of the allophenic mice is noteworthy, as the mice were produced by injecting blastocysts with malignant teratocarcinoma stem cells. These were from two separate transplant lines: One (OTT 6050) was an X/Y euploid tumor line of the 129 strain, the other (72484-395) an X/X euploid line of the LT strain (Cronmiller and Mintz, 1978). Despite the lack of tumors, even in an animal as old as 89 weeks of age, tumor-strain cells were directly visualized *in situ* in tissue sections, along with blastocyst-derived cells. The respective tumor-derived and blastocyst-derived cell strains, identifiable by quantitative strain differences in BGS activity, were readily recognizable as comparably differentiated cells. The mosaic tissues were indistinguishable in structure and differentiation from the corresponding pure-strain tissues of controls.

Normalcy of tumor-lineage cells has been verified in a much larger number of experimental animals obtained in this laboratory from teratocarcinoma-injected blastocysts (Custer and Mintz, manuscript in preparation). The results, from strain-specific isozyme analyses, supplemented by histological study, show that if the teratocarcinoma stem cell is integrated into the embryo and becomes normalized, its mitotic descendants participate in embryogenesis and give rise only to differentiated normal cells that synthesize adult products (hemoglobins, immunoglobulins, etc.) (Mintz and Illmensee, 1975; Illmensee and Mintz, 1976) and remain normal. If the cells are not successfully integrated, they continue to be malignant and form tumors—all of them *teratocarcinomas*—as if in a culture chamber. One case at first thought to be another tumor, a presumed "pancreatic adenocarcinoma" was reported earlier (Illmensee and Mintz, 1976). This turned out, upon further study, to be a "cryptic" teratocarcinoma that was, misleadingly, embedded in the pancreatic acinar tissue of the host. That host had no other cells derived from the tumor. The tumor itself lacked pancreas-specific enzymes (Filler and Mintz, unpublished data) and formed typical teratomatous embryoid bodies when injected in the body cavity of a syngeneic recipient. Thus, the present *in situ* histochemical data (Figs. 1–3), reinforced by our previous observations, strongly support the conclusion that completely normal and stable differentiation has been elicited in malignant teratocarcinoma stem cells after they have been placed in the company of normal embryo cells. The mechanisms of normalization remain obscure, although one may presume that cell-surface interactions are likely to be initially critical in the process (Mintz, 1977, 1978).

The present report also describes the applicability of the BGS histochemical marker to *in situ* genotypic analyses of tissues such as pancreas, kidney, and salivary gland (Figs. 2–4) in allophenic mice. Previously, only its use in brain (Fig. 1) has been reported (Dewey *et al.,* 1976). The patterns observed here in mice produced from teratocarcinoma cell-injected blastocysts are reproducible among the experimental animals (cf. Figs. 1c and d, and Figs. 3c and d). They are also indistinguishable from the patterns visualized in those tissues of conventional allophenic mice produced from all-normal cells, i.e., from aggregated blastomeres, as seen thus far either with the BGS marker in brain (Dewey *et al.,* 1976) or with the $\beta$-glucuronidase histochemical marker in some of the other tissues (Condamine *et al.,* 1971; Mintz, unpublished data). Therefore, as one would expect, the histogenesis of specific tissues is the same, whether comprised partly of normalized teratocarcinoma-lineage cells or entirely of cells of embryonic provenance.

In the Purkinje layer of allophenic cerebellum, the two genotypic classes of cells are closely intermingled (Figs. 1c and d). As already stated in a study of other allophenic mice (Dewey *et al.,* 1976), this is not surprising, as extensive cell migrations and rearrangements are known to be involved in cerebellar histogenesis.

In the exocrine pancreas (Figs. 2c and d), the partitioning off, by connective tissue septa, of large single-genotype patches of cells suggests quite another mode of histogenesis: clonal expansion of sheets of acinar precursor cells within the developing barriers of the connective tissue framework, with little or no autonomous cell migration.

In kidney convoluted tubules as well (Figs. 3c and d), those high-activity cells that are most confidently identified are invariably found in relatively large clusters comprising several neighboring tubules. Kidney development therefore also does not consist of fine-grained interspersion or migration of cells.

Of course, at the borders between genotypic patches in any tissue there are always irregularities and intermingling of single cells or small groups of cells of the respective strains, as first described in the clones of melanocytes in the coat (Mintz, 1974). However, our observations do not justify the emphasis placed by Feder (1976) on "solitary" cells in most tissues. Short-range intermixing, at the margins of two mitotically expanding neighboring populations competing for available space, is merely a natural corollary to the gradual expansion of relatively coherent cellular masses in such coarse-grained tissues.

In our preliminary explorations of pure-strain tissues, to determine whether the BGS marker was applicable, several unexpected observations were made (Dewey and Mintz, 1978). These, briefly referred to here, indicate that the known genetic categories of BGS, based on activity of the enzyme in brain (Felton *et al.,* 1975), are insufficient to foretell if BGS can be exploited as a marker in other tissues: First is the fact that some tissues within a strain may show a quantitative reversal of BGS activity (Table 1) in relation to activity in brain. Second, two cell types within a tissue (e.g., exocrine vs endocrine pancreas cells) may have independent and disparate trends in BGS activity levels. In addition, the cell types within a tissue are highly heterogeneous in BGS levels and only some cell types (e.g., Purkinje cells in cerebellum) have sufficient enzyme activity in the high-level strain to be valid candidates for studies with the marker.

The BGS histochemical marker has proved to be useful in directly visualizing cellular genotypes in tissues from mice derived from teratocarcinoma-injected blastocysts. Both strain lineages have now been revealed *in situ* in various tissues. In one, the Purkinje cells of the brain, in mouse No. 1 (Fig. 1c), those cells are clearly derived *almost entirely from the tumor.* Yet the marker has documented their normalcy and fully differentiated status. It has also contributed information on their histogenesis.

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