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# Mosaic mice with teratocarcinoma-derived mutant cells deficient in hypoxanthine phosphoribosyltransferase

(Lesch–Nyhan disease/cell selection/trisomy/allophenic mice/blastocyst injection)

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ABSTRACT **Mutagenized stem cells of a cultured mouse teratocarcinoma cell line were selected for resistance to the purine base analog 6-thioguanine. Cells of a resistant clone were completely deficient in activity of the enzyme hypoxanthine phosphoribosyltransferase (HPRT, IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8), the same X-linked lesion as occurs in human Lesch–Nyhan disease. After microinjection into blastocysts of another genetic strain, the previously malignant cells successfully participated in normal embryogenesis and tumor-free, viable mosaic mice were obtained. Cells of tumor lineage were identified by strain markers in virtually all tissues of some individuals. Mature function of those cells was evident from their tissue-specific products (e.g., melanins, liver proteins). These mutagenized teratocarcinoma cells are therefore developmentally totipotent. Retention of the severe HPRT deficiency in the differentiated state was documented in extracts of mosaic tissues by depressed specific activity of the enzyme, and also by presence of unlabeled clones in autoradiographs of explanted cells incubated in [$^3$H]hypoxanthine. Some mosaic individuals had mutant-strain cells in only one or a few tissues. Such animals may provide unique opportunities to identify the tissue sources of particular aspects of the complex disease syndrome. The tissue distribution of HPRT-deficient cells suggests that selection against them is particularly strong in blood of the mosaic mice, as is already known to be the case in human heterozygotes. This phenotypic parallelism supports the expectation that afflicted $F_1$ male mice that might be obtained from mutant germ cells can serve as a model of the human disease.**

The embryonal stem cells in transplantable mouse teratocarcinomas may continue to proliferate and also to differentiate into a disorganized, limited assortment of tissues (1, 2). When stem cells from an *in vivo* transplanted tumor (OTT 6050) were microinjected in small numbers (1–5 cells) into early embryos at the blastocyst stage, they underwent normal, stable differentiation (3–5). Healthy mosaic mice with both tumor-derived and embryo-derived functional somatic tissues were obtained. Functional germ cells were also sometimes formed from this chromosomally X/Y tumor, in males, and gave rise to normal $F_1$ progeny. The conclusion was reached that the stem cells of that tumor line had retained the normal genetic complement and were developmentally totipotent. Whether they behaved in a normal or a malignant fashion was a matter of change in gene expression rather than gene structure, and the choice was determined by local environmental influences.

The above tests for totipotency of "wild-type" teratocarcinoma cells were undertaken in part to establish the feasibility of converting *mutant* teratocarcinoma cells into mice bearing specific mutations (6, 7). Gametes or fertilized eggs, from which development ordinarily occurs, are not self-propagating in culture and therefore cannot yield clones in which a desired mutation may be selected following exposure to a mutagenic agent. Because teratocarcinoma cells are in fact developmentally totipotent, their adaptability to culture makes them promising vehicles for the deliberate introduction of specific mutant genes into mice.

One of the possible uses of such an experimental system would be the construction of animal models of human genetic diseases (6, 7). An example is the Lesch–Nyhan syndrome (8), due to a severe deficiency of hypoxanthine phosphoribosyltransferase (HPRT, IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) (9), which functions in the salvage pathway of purine biosynthesis. The defective (recessive) form of the X-linked gene leads, in affected males, to excess formation of uric acid and to severe neurological symptoms, including self-mutilation, spasticity, and mental retardation (10). The HPRT gene is apparently also X-linked in the mouse (11), but no mutation has yet been detected in the laboratory species.

We report here that cells from an HPRT-deficient *in vitro* mouse teratocarcinoma stem-cell line have proven to be normalizable and developmentally totipotent when injected into genetically marked blastocysts. In the mosaic mice that have resulted, mature functional cells of the tumor strain have retained the HPRT deficiency and are found in essentially all tissues of some animals. In other individuals, only one or a few tissues have cells derived from the mutant cell lineage; these cases may help to identify the tissue sources of particular aspects of the disease.

## MATERIALS AND METHODS

**Teratocarcinoma Cell Line.** The wild-type starting material was the PSA 1 teratocarcinoma cell line (refs. 12 and 13; unpublished data) clonally derived from primary cultures of the OTT 5568 mouse teratocarcinoma; that tumor was produced (14) almost a decade ago by the method of grafting a blastocyst-stage embryo of the *agouti black* 129/Sv *Sl$^J$ C P* inbred strain (to be referred to as 129) under the testis capsule of an adult (Fig. 1). PSA 1 cells were maintained on a mitomycin C-treated feeder layer (12) of the mouse embryo fibroblast STO cell line (from Alan Bernstein of the Ontario Cancer Institute). STO cells are 6-thioguanine-resistant. To obtain mutant cells, PSA 1 cultures were exposed for 2 hr to medium containing 20 $\mu$M *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and then seeded at $10^7$ cells per 10-cm$^2$ confluent STO feeder layer. After growth for 7 days in the absence of a selective agent, to allow for expression of mutant phenotypes, HPRT-deficient mutants

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Abbreviations: HPRT, hypoxanthine phosphoribosyltransferase; APRT, adenine phosphoribosyltransferase; GPI, glucosephosphate isomerase; MUP, major urinary protein complex.

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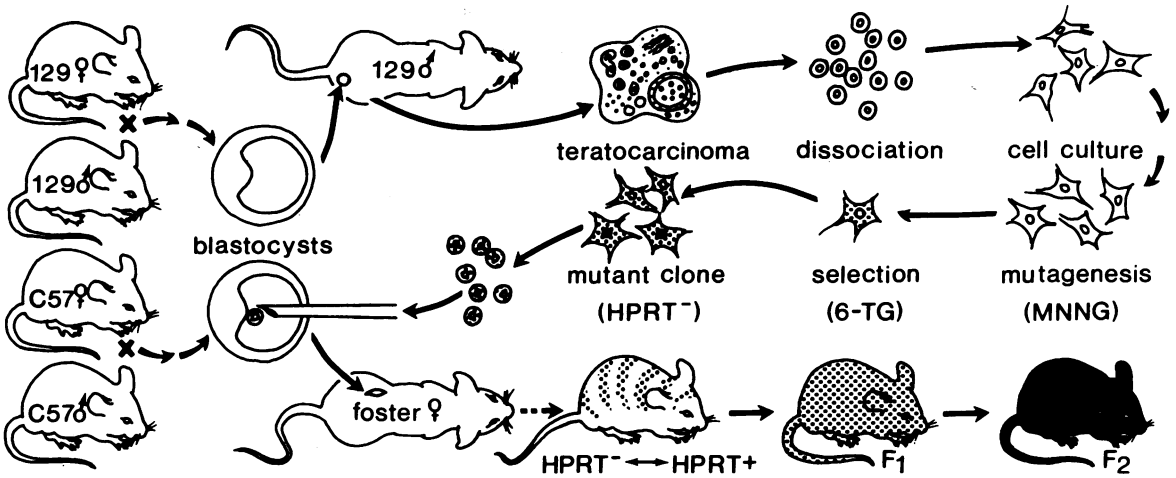


FIG. 1. The plan of the experiment, spanning almost a decade, is diagrammed, starting at the upper left. A blastocyst from a mating of 129-strain mice was grafted under the testis capsule of a syngeneic host (14). The graft formed a malignant teratocarcinoma. After dissociation, tumor cells were explanted and the stem cells were established as an *in vitro* culture line (ref. 12, and unpublished data). Following exposure to the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), HPRT⁻ cells (stippled) were selected for their resistance to 6-thioguanine (6-TG). Cells from a resistant *clone* were then microinjected into the cavity of genetically marked blastocysts (e.g., of the C57BL/6 strain). The injected embryos were transferred to the uterus of a pseudopregnant foster mother (previously mated to a sterile vasectomized male) and live mice were born. Some were mosaics comprising HPRT⁻ cells derived from the mutant teratocarcinoma lineage, along with blastocyst-derived cells, in their coats (striped) and/or internal somatic tissues. Tissue-specific effects of the deficiency are analyzable in the mosaics. If mutant cells are of X/O (as in this case) or X/X sex chromosome type and contribute to the germ line of females, affected "Lesch–Nyhan" males would be obtained in the $F_1$ generation; if the mutant cells are X/Y, in mosaic (non-Lesch–Nyhan) males, affected males would occur in the $F_2$. (Based upon figure 17, ref. 6.)

were selected in medium containing 0.1 mM 6-thioguanine. One of the resistant clones, NG 2, was used for blastocyst injections.

**Blastocyst Injections.** The microinjection procedure was as previously described (4). Three to five NG 2 cells were introduced into the cavity of blastocysts that had numerous genetic markers distinguishing them from the 129 tumor strain. Blastocysts were from ICR sublines of the following inbred strains: C57BL/6 (C57), WH, CBA with the T6/T6 homozygous translocation, and $F_1$ (CBA-T6/T6 × C57) hybrids; in some cases, color-marked *brown* (*b/b*) or *beige* (*bg$^J$/bg$^J$*) strains coisogenic with C57 were used. Injected blastocysts were surgically transferred to uteri of pseudopregnant ICR randombred females, for development to term.

**Analyses of Tissue Genotypes.** All animals were screened at 7–10 days for coat mosaicism and at approximately 4 weeks for blood and liver mosaicism. Blood-cell lysates were analyzed for strain-specific electrophoretic variants of glucosephosphate isomerase (GPI; D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9), as described elsewhere (15). Liver genotypes were revealed by electrophoretic patterns (16) of proteins normally excreted in the urine (major urinary protein complex, MUP) but synthesized in the liver (17) and thus indicative of hepatic parenchyma genotypes (18). At autopsy, blood and soft tissues were frozen in dry ice, stored at −70°, and tested for GPI isozymes.

**HPRT Specific Acitvity.** Tissue homogenates of some experimentally derived mice were thawed, immediately sieved on columns of Sephadex G-25, and assayed as coded specimens for HPRT and also for adenine phosphoribosyltransferase (APRT, EC 2.4.2.7) activity, as previously described (19, 20), except that 4 mM thymidine triphosphate was present in all assays. The assays were linear with both time and protein concentrations; the latter were estimated (21) with bovine serum albumin as a standard.

**Autoradiography.** Skin and subcutaneous connective tissue from two mice with coat mosaicism were explanted. The fibroblastic cell cultures were incubated in [$^3$H]hypoxanthine at 2.5 μCi/ml (0.35 μM) for 14 hr, followed by standard methanol/acetic acid fixation and processing. Slides were coated with Kodak NTB-2 emulsion, exposed in the dark for 7 days at 4°, developed, and stained with May–Grünwald–Giemsa.

## RESULTS

Extracts prepared from thioguanine-resistant cultured NG 2 teratocarcinoma cells showed no detectable HPRT activity. Tests aimed at discriminating between presence of enzymatically inactive HPRT protein and actual loss of HPRT—hence possible deletion of the locus—are inconclusive to date.

Forty-four mice were obtained from injected blastocysts. Included were 21 females, 22 males, and 1 (living) male with possible intersexuality. Twelve animals were autopsied between the ages of 3 days and 6 weeks. Of the 32 mice still alive, the oldest are 9 months of age; all appear healthy and have no visible tumors.

Among the 12 animals autopsied, as many as nine (cases 1–9) had teratocarcinoma-derived cells in one or more of their tissues, as evidenced by 129-strain-specific markers (Table 1). Among the 32 living animals, in which only the coat, blood, and liver have been tested, one (case 10) has liver mosaicism; and the abnormal male (not listed) is likely to be a sex chromosome mosaic (22, 23). Some of the remaining 30 mice may prove to have 129-strain cells in some internal tissues.

In the most striking case (case 1, Table 1), 18 tissues (including hair follicles as well as melanocytes in the coat) were genotypically analyzed and, with the notable exception of blood, all contained 129-strain cells. Examples are seen in Fig. 2 *left*, with the almost-ubiquitous GPI isozyme marker. In another animal with extensive mosaicism (case 2, Table 1), 15 tissues were analyzed and 13 of them—again excluding blood, and also salivary glands—had some cells of the tumor strain.

Evidence for normal specialized tissue functions of 129-strain cells was found in hair follicles, with the phaeomelanin (*agouti* locus) discriminant (Table 1, cases 1 and 2); in melanocytes, with the 129 *non-beige* eumelanin marker (case 1); and in

Table 1. Percent tissue contributions* derived from HPRT-deficient teratocarcinoma cells (129 strain) after injection into blastocysts (C57BL/6 strain)

| Case, sex | Autopsy age | Coat | Blood cells | Liver | Spleen | Thymus | Heart | Lungs | Kidneys | Gut | Pancreas | Salivary glands | Muscle | Brain | Gonads | Repr. tract† |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 ♀‡ | 5 weeks | 5 | 0 | 15 | 10 | 30 | 55 | 5 | 50 | 40 | 5 | 10 | 25 | 10 | 25 | 20 |
| 2 ♂ | 2 weeks | 5 | 0 | 30 | 10 | 10 | 50 | 10 | 20 | 40 | 5 | 0 | 40 | 15 | 15 | 25 |
| 3 ♀ | 3 days | | 0 | 5 | 15 | 0 | 60 | 10 | 40 | 20 | 0 | 0 | 20 | 0 | 20 | 20 |
| 4 ♂ | 6 weeks | 0 | 5 | 10 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 15 |
| 5 ♂ | 5 days | | | 0 | 0 | 0 | 20 | 0 | 5 | 0 | | 0 | 0 | 0 | 0 | 0 |
| 6 ♂ | 3 weeks | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 |
| 7 ♀ | 3 weeks | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 |
| 8 ♀ | 3 weeks | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 |
| 9 ♂ | 3 weeks | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 |
| 10 ♂ | Alive | 0 | 0 | 5§ | | | | | | | | | | | | |

* Strain-specific markers in the coat were at the *A* (*agouti*) locus, expressed in hair-follicle dermis, in all; in addition, gene substitutions at single pigmentary loci supplied melanocyte markers in cases 3, 5, 6, 7, 9, 10 (*b*/*b* blastocysts) and 1, 4 ($bg^J/bg^J$ blastocysts). Other tissue analyses were based on GPI isozymic strain differences.

† Accessory reproductive glands and ducts.

‡ Additional GPI analyses revealed a 15% 129-strain tissue contribution in adrenals and 10% in skin.

§ Liver genotypes in *living* animals were determined by the MUP marker in urine samples.

hepatocytes, with the MUP strain variant (case 10; Fig. 2 *right*). In addition, a normal developmental feature unique to myogenesis was seen in muscle: an intermediate or heteropolymeric GPI band (cases 1–3, Table 1; Fig. 2 *left*) representing heterokaryon formation by fusion of uninucleated myoblasts (24).

Progeny tests of living animals, to detect any germ-line transmission from the tumor strain of origin, are still incomplete. Karyotype analyses show that the NG 2 cell line has a modal class (72%) of 40 chromosomes; the sole apparent anomalies are trisomy of chromosome 6 and presence of only one sex chromosome, i.e., a single X (C. Cronmiller and B. Mintz, unpublished). If X/O cells from the NG 2 line are in fact able to contribute germ cells *in vivo*, these should become female rather than male gametes: The Y chromosome is needed for maleness, the X/O mouse being a fertile female (25); and functional reversal of germ cells of one sex chromosome type to the opposite sex phenotype does not occur or is exceedingly rare in allophenic mice (22).

Among the known mosaics already autopsied, three (cases 1–3, Table 1) have some tumor-strain cells in their gonads. Two are phenotypic females with some 129-strain ovarian cells (Fig. 2) and one is a male with some testicular cells of that strain. The gonadal 129-type cells may have been germinal and/or somatic and, if germinal, might have become functional eggs in the females.

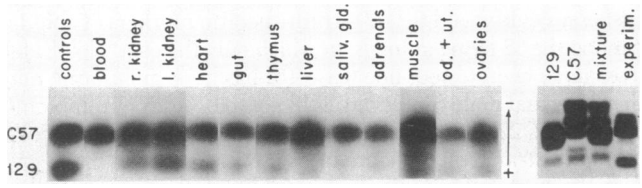


FIG. 2. (*Left*) Glucosephosphate isomerase allelic strain variants in starch gel electrophoresis of tissue extracts from female mosaic *mouse no. 1*. The HPRT-deficient teratocarcinoma cell strain (129) is absent from its blood but present in all other tissues (including five additional tissues listed in Table 1 but not included here). Three bands in muscle denote normal myoblast fusion leading to heterokaryons. Note the 129-strain cells, which are chromosomally X/O female, in the ovaries. (od. + ut., oviduct plus uterus.) (*Right*) Acrylamide gel electrophoretic patterns of allelic strain variants of the major urinary protein complex in experimental *mouse no. 10*, and controls. A 129-strain contribution in the mosaic indicates that normally functioning parenchyma cells of that strain are present in its liver, where the protein is produced.

In order to learn whether the 129-strain cells in differentiated tissues of mosaic animals had continued to be HPRT-deficient *in vivo*, two kinds of assays were conducted. The first measured HPRT specific activity of tissue homogenates after their 129-type tissue contribution had been ascertained from GPI isozyme tests. HPRT activity was expressed in relation to APRT activity, because the latter is unaffected in thioguanine-resistant cells of non-erythroid tissues of Lesch–Nyhan patients (10). All samples were encoded before HPRT and APRT assays. In the experimental animal designated case 1 (Table 2), there is a clear correlation between the presence of an appreciable proportion (50–55%) of putatively $HPRT^-$ (129-strain GPI type) cells in heart and kidney and a marked depression of the HPRT/APRT ratios in those tissues, as compared with $HPRT^+$ control tissues of the blastocyst and tumor strains. But when only minor amounts (5–10%) of 129 cells occurred, as in the brain and pancreas of case 1, no significant lowering of the ratio was seen. In case 2, results for three of the five tissues (heart, pancreas, and salivary gland) substantiated these trends.

Independent confirmation was obtained by autoradiographic

Table 2. HPRT specific activity* in tissues of mice partially derived from HPRT-deficient teratocarcinoma cells

| Tissue | HPRT/APRT C57 control | HPRT/APRT 129 control | HPRT/APRT Exp.‡ | % 129-type GPI† |
|---|---|---|---|---|
| Heart§ | 3.08 | 1.90 | 0.50 | 55 |
| Kidney§ | 0.79 | 1.01 | 0.48 | 50 |
| Brain | 1.58 | 1.79 | 1.81 | 10 |
| Pancreas | 0.56 | 0.58 | 0.48 | 5 |
| Heart§ | 0.54 | 0.52 | 0.26 | 50 |
| Liver | 0.57 | 1.25 | 0.51 | 30 |
| Spleen§ | 0.73 | 0.78 | 0.42 | 10 |
| Pancreas | 0.22 | 0.28 | 0.25 | 5 |
| Salivary gland | 0.36 | 0.19 | 0.29 | 0 |

* The standard deviations of the specific activities of the indicated (§) samples were less than 20% ($n = 8$ determinations). Tissue extracts from the two experimental animals, and their respective controls, were stored frozen for two different periods of time prior to assay; this accounts for the different ratios for heart and pancreas in the two experiments.

† The teratocarcinoma strain contribution; data from Table 1.

‡ The experimental animal in the upper section is case 1 (Table 1); the one in the lower section is case 2.

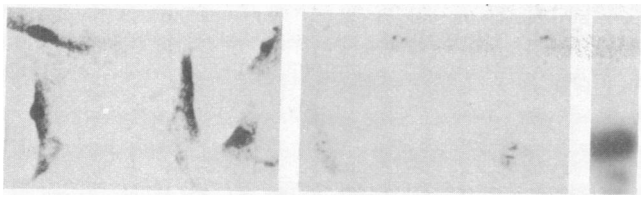

FIG. 3. In a culture of fibroblastic cells grown from subcutaneous connective tissue of *mouse no. 2*, a part of a normal colony (*Left*) and of a markedly HPRT-deficient colony (*Center*) are seen in autoradiographs after [$^3$H]hypoxanthine labeling. (× 126.) The GPI starch gel at the *Right* confirms the presence of a majority of the fast-moving (C57, normal) and a minority of the slow-moving (129, HPRT$^-$) cell strains in a lysate of the same culture.

visualization of HPRT$^-$ colonies in cultures of subcutaneous connective tissue taken from mouse no. 2 at autopsy. The cells were plated at low density, cultured for 1 week, and incubated 14 hr in [$^3$H]hypoxanthine. The autoradiographs (Fig. 3 *left* and *center*) clearly showed two kinds of colonies: Some had many silver grains over the cells, as in wild-type (HPRT$^+$) control cultures; others were virtually devoid of grains, as in the HPRT$^-$ STO cultures. The low frequency of the unlabeled colonies from the mosaic animal conforms to the minor representation of the slow-migrating 129-type of GPI in a cell extract of the same culture (Fig. 3 *right*).

## DISCUSSION

When mouse teratocarcinoma cells with severe HPRT deficiency—comparable to that in human Lesch–Nyhan disease (8, 9)—are introduced into wild-type blastocysts (Fig. 1), they cease to be malignant and proceed to contribute to normal embryogenesis. In the viable mice thus obtained, normally functioning differentiated cells of the tumor strain have been found, by means of strain-specific markers, in virtually all tissues of some individuals. The stem cells of this *in vitro* line, known as NG 2, are therefore developmentally totipotent.

Maturation *in vivo* of cells from any teratocarcinoma line initially characterized by a biochemical lesion does not in itself ensure that the lesion is still present many cell generations later in the differentiated population. Assays of HPRT specific activity were therefore carried out and did in fact document retention of the deficiency (Table 2): When appreciable populations of NG 2-derived cells were present, a marked depression of HPRT activity was found. In addition, autoradiographs of [$^3$H]hypoxanthine-incubated cultures from connective tissue of mosaics showed some unlabeled (HPRT$^-$) and some labeled (HPRT$^+$) colonies (Fig. 3). The HPRT change in NG 2 thus appears to constitute a bona fide mutation. Germ-line transmission would afford final proof.

The present experiment initiates the experimental application of a scheme previously proposed (6, 7) for analyzing mammalian differentiation and disease. The objective is to select *in vitro* for specific mutations in developmentally totipotent cells, so that the effects of a biochemically defined change may then be followed *in vivo* at all levels of biological organization. With this system, mouse models of human genetic diseases might be produced (6, 7). Lesch–Nyhan disease was chosen as the first candidate partly because of the ease with which the relevant X-linked enzymatic lesion (9) is selectable in culture. While the actual model, an $F_1$ "Lesch–Nyhan" male mouse, is not yet on hand, the mosaics themselves (both females and males) have certain unique and useful features not available in "models" whose genetic lesion is in all their cells. As seen in Table 1, a given mosaic animal may have HPRT$^-$ cells in only a single tissue, such as brain (cases 7–9), or in only a few tissues,

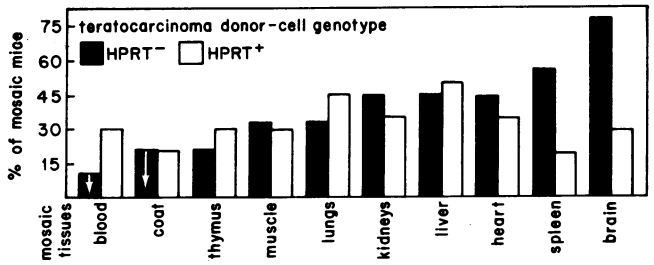


FIG. 4. Percent *of mosaic mice* with mosaicism of each of the tissues indicated. Data are based on 9 mosaics with HPRT$^-$ cells (derived from the OTT 5568 teratocarcinoma; this series) and 20 mosaics with HPRT$^+$ cells (derived from the OTT 6050 teratocarcinoma; refs. 4 and 5; further unpublished data). Only mosaic animals with virtually all tissues genotypically analyzed are included. The definitive frequency of blood and of coat mosaicism in the final population of HPRT$^-$ mosaics is certain to be *less* than shown, as designated by downward arrows (see *text*). Results suggest specific selection against HPRT$^-$ cells in blood.

such as kidneys and heart (case 5). The metabolic and clinical attributes of such individuals could help to identify a tissue in which the complex syndrome of the disease may originate. No obvious behavioral defects have been seen in the mosaics. This might signify that the brain, despite its very high normal levels of HPRT (10), is surprisingly little dependent on the salvage pathway of purine biosynthesis; or that the brain regions where these HPRT$^-$ cells (a small minority, in any case) are located are not crucial to the behavioral manifestations in affected individuals; or that systemically distributed products from other tissues are responsible for secondary effects on the nervous system. The deleterious accumulation of excessive purine degradation products is of course a major characteristic of the disease. The mosaic mice may also help to define the chief tissue sources of that problem.

Some preliminary indications of tissue-specific phenotypic effects are already detectable through cell selection in the mosaic animals. The first of these, in the blood, is of interest because it parallels selective trends in human HPRT$^+$/HPRT$^-$ heterozygotes. In those females, there is cellular phenotypic, but not genotypic, mosaicism owing to expression of only one X-linked allele per cell. Another difference as compared to HPRT$^+$ ↔ HPRT$^-$ mice with cellular genotypic mosaicism is that the two phenotypic heterozygous populations of cells are probably approximately equal early in development, whereas sporadic incorporation of teratocarcinoma-derived cells may result in their scattered representation in tissues of allophenic mice (Table 1). The human deficiency may be severe or moderate; though heterozygous carriers of the severe type have a normal and a deficient class of fibroblasts (26), they have normal erythrocytes (27). Only carriers of the moderate deficiency have individual erythrocytes of the two separate phenotypes (28). Thus, in human erythrocytes, HPRT deficiency is critical at the cellular level and causes a greater competitive disadvantage as it becomes more severe. Carriers of the gross deficiency do, however, have some HPRT$^-$ lymphocytes (29).

These two trends appear to be mirrored in our experimental mice. In Fig. 4, the frequency with which each tissue has any teratocarcinoma-strain cells is compared in the mosaic mice of two groups: the present one, in which the cells of donor origin are HPRT$^-$ (9 mosaics; Table 1), and an earlier group, in which they are HPRT$^+$ (20 mosaics; refs. 4 and 5, and further unpublished data). The data from the HPRT$^-$ experiment are arranged (left to right) from the least frequently mosaic tissue, blood (11%), to the most frequently mosaic, brain (78%). However, the *final* frequency in this experiment is certain to

diminish still further in blood (and coat): When all living mice have been autopsied and analyzed, no new blood mosaics will have been identified, but the size of the mosaic population will have increased by at least one (case 10). As seen in Fig. 4, teratocarcinoma-derived cells are less often found in blood if the cells are $HPRT^-$ than if they are $HPRT^+$. On the other hand, in the spleen, which is composed largely of lymphocytes, $HPRT^-$ cells are frequently included (56% of mosaic mice). Though other factors (e.g., origin of $HPRT^-$ and $HPRT^+$ cells from separate tumors, or trisomy-6, or hidden mutations in NG 2) may play a role in cell selection, the parallelism to the selective trends in human heterozygotes is an encouraging indication that the "synthesis" of mice with $HPRT^-$ cells, whether in some or all of their tissues, will be a useful way to examine the basis for the human disease, and possibly to attempt its cure.

While the brain is most frequently mosaic, it has one of the lowest levels of $HPRT^-$ cells (Table 1, Fig. 4). Therefore, selection in the brain may involve two steps: first, relative ease of entrance of $HPRT^-$ cells into the precursor pool; second, relative discrimination against those cells as brain development progresses.

Of the two karyotype changes in NG 2 cells (C. Cronmiller and B. Mintz, unpublished), the occurrence of a single X chromosome and no Y is not necessarily disadvantageous, because X/O mice are viable fertile females (25). However, trisomy-6, like all autosomal trisomies of mice, is usually lethal prenatally. It leads (in non-mosaic embryos) to retardation and hypoplasia, and death between 12 and 14 days of gestation (30). The full functional maturation of cells of trisomic lineage in the animals described here implies that this trisomy is not a *cell* lethal.

Animals whose tissues comprise NG 2-derived cells now range up to 9 months of age and have remained free of tumors. This reinforces our earlier observations following injections of small numbers of cells from another tumor line (OTT 6050), in which very few animals developed teratomas (refs. 3–5, and further unpublished data). Those tumors apparently arose only from teratoma cells that failed to become integrated into the embryo: When single cells were injected into blastocysts, mutually exclusive results were obtained, leading either to participation in normal differentiation or to retention of teratomatous growth. In the former cases, mosaic normal tissues remained normal, even if transplanted to new hosts. Thus, induction of normal differentiation of teratocarcinoma stem cells in blastocysts stably terminated their malignancy.

The teratocarcinoma cell line used in the present experiment was *clonally* derived; it exhibited typical malignant behavior in subcutaneous grafts. The stem cells introduced into blastocysts therefore *all* presumably possessed malignant potential yet were successfully normalized.

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