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X-Linked Hypoxanthine-Guanine Phosphoribosyl Transferase Deficiency: Heterozygote Has Two Clonal Populations

Abstract. *Clones of skin fibroblasts cultured from the mother of two sons with X-linked hypoxanthine-guanine phosphoribosyl transferase deficiency (Lesch-Nyhan syndrome) were assayed for activity of this enzyme by measurement of the incorporation of ^{3}H-guanine into guanylic acid as counts per minute per microgram of protein and by autoradiography. The demonstration of two populations of clones, wild-type clones with normal enzyme activity and mutant clones unable to incorporate ^{3}H-guanine, is evidence that the locus for hypoxanthine-guanine phosphoribosyl transferase on one of the X chromosomes is inactive.*

A familial syndrome consisting of hyperuricemia, mental retardation, choreoathetosis, and compulsive self-mutilation was described by Lesch and Nyhan (*1*). There is substantial evidence that the mutation is X-linked, since transmission of the disorder, which occurs only in males, is from mother to son (*2*). The disease is associated with absence of activity of an enzyme involved in purine metabolism, namely, hypoxanthine-guanine phosphoribosyl transferase (HGPRT) (*3*).

As determined by autoradiography, some skin fibroblasts from the mother of an affected male incorporate ^{3}H-hypoxanthine into RNA, an indication of phosphoribosyl transferase activity, whereas other cells do not (*4*). This finding is interpreted as support for the hypothesis of inactivation of one X chromosome in female somatic cells (*5*). The demonstration of a mosaic pattern in the fibroblasts grown from the mother of an affected child is compatible with inactivation of the HGPRT locus in one X chromosome; however, a critical test of X inactivation at this locus is the examination of HGPRT activity in clones cultured from a heterozygote. We have obtained clones of fibroblasts from the mother of two boys with Lesch-Nyhan syndrome (HGPRT deficiency) and have examined them quantitatively and autoradiographically for their ability to incorporate ^{3}H-guanine into guanylic acid.

Fibroblast cultures were established from skin biopsies from M.W., one of the two brothers described in the original report (*1*), and from his parents. Two biopsy specimens were obtained from the mother, one from each forearm. Cells were grown in minimum essential media enriched with 1 percent nonessential amino acids, 5 percent beef embryo extract ultrafiltrate, and 20 percent human A serum and maintained in a 5 percent CO_2 atmosphere at 37°C. Clones were obtained according to techniques of Ham and Puck (*6*). Cells in suspension after dissociation by treatment with trypsin were single when plated, and clones were plucked only if they were well isolated from neighboring clones. Each uncloned fibroblast culture and clone were assayed for HGPRT activity in two ways.

In the first method cells in logarithmic phase growth were subcultured into bottles with 15 to 20 ml of growth medium. The cell suspension was transferred into three or four Falcon plastic 60-mm petri dishes so that, for each clone or for each uncloned specimen, cultures were established at least in triplicate. After 24 to 48 hours at 37°C, the cells were incubated for 1 hour with ^{3}H-guanine (0.3 c/mmole) in a final concentration of 1 μc/ml of medium. Petri dishes were then washed twice in saline (10 minutes for second saline wash) and fixed in 95 percent ethanol. The cells were removed from petri dishes with rubber policemen, suspended in 1 ml of 0.9 percent NaCl, and disrupted by sonic vibration. The resulting cell extracts were assayed for protein concentration (*7*) and uptake of ^{3}H-guanine. The amount of radioactivity was determined on 0.6 ml of the cell extract in 10 ml of Bray's solution by means of a Packard liquid scintillation counter. The control samples (t_0) were replicate cultures to which ^{3}H-guanine was added and immediately removed by washing as previously described.

In the second method, triplicate cultures established in 35-mm Falcon plastic petri dishes were allowed to grow for 24 to 48 hours in 5 percent CO_2 before incubation with ^{3}H-guanine (9.8 c/mmole) in a final concentration of 2.5 μc/ml medium. After 6 hours of incubation at 37°C, the cells were washed in saline, fixed in ethanol, extracted with 5 percent cold trichloroacetic acid, washed in water, and dried. Petri dishes were dipped in Kodak NTB-2 liquid emulsion, and after exposure for 1 week, the autoradiographs were developed, fixed, and washed; they were examined by both phase and bright-field microscopy. Petri dishes were scored as positive when cells were labeled with ^{3}H-guanine, and negative only when all of the cells were unlabeled and visible only with phase microscopy. Each specimen was scored without knowledge of its source.

Table 1. HGPRT activity determined with ^{3}H-guanine as counts per minute per microgram of protein. Each value used to calculate the mean represents the average of the triplicate determinations. Negative clones have deficient HGPRT activity; positive clones have normal HGPRT activity.

Source of fibroblasts	Cultures examined	Clones (No.)	Mean (cpm/μg protein)	Range or standard deviation
Father	Uncloned		12.22	2.91 to 29.99
Affected son	Uncloned		.16	0.8 to .20
Mother, biopsy No. 1	Uncloned		3.43	3.42 to 3.44
Mother, biopsy No. 1	Negative clone	10	.12	± .09
Mother, biopsy No. 1	Positive clone	10	4.72	±1.75
Mother, biopsy No. 2	Uncloned		11.58	7.22 to 15.93
Mother, biopsy No. 2	Negative clone	8	.19	± .18
Mother, biopsy No. 2	Positive clone	20	10.84	±7.13

Since HGPRT is essential for the phosphorylation of guanine, mutant cells without the enzyme would be unable to incorporate ^{3}H-guanine into guanylic acid and therefore, would show radioactivity equal to that in controls (t_0) and appear unlabeled in autoradiographs.

The father's fibroblasts (Table 1) had HGPRT activity in contrast to those of his affected son which had activity in the range of background and not significantly greater than that of the t_0 specimens. Uncloned specimens from the mother had activities between that of the mutant and the father. The second specimen had consistently greater activity than the first. Cloning efficiency of maternal cells was from 20 to 40 percent. Two distinct clonal populations were found, one which had the same HGPRT activity as the son's mutant cells and one which had from 10 to 200 times the activity of the mutant cells (Table 1).

Each petri dish, as examined with phase-contrast objectives, had abundant cells for analysis. Since the cells were unstained, they were not visible under bright-field objectives unless labeled with ^{3}H-guanine. When labeled, the cells showed heaviest labeling in the nucleus and least in the cytoplasmic processes. All cells from the father incorporated ^{3}H-guanine, although there were obvious differences in the amount of label from cell to cell and occasionally unlabeled cytoplasmic processes. In the mother's uncloned specimen No. 1 there were occasional unlabeled cells, but not as many as one might expect considering the number of negative clones obtained from this specimen. The mother's second specimen, with nearly normal quantitative values, had only rare unlabeled cells. Petri dishes of cloned cells were either labeled or completely unlabeled (Fig. 1). These results correlated exactly with the amount of activity determined by direct scintillation counting for the same clone. That is, a negative clone determined quantitatively was also negative as determined by autoradiography (Table 1).

The mother, who is clinically normal, is an obligate heterozygote since she has given birth to two affected sons. Our results demonstrate that she has two clonal populations of fibroblasts in her skin, wild-type clones with normal HGPRT activity and mutant clones lacking this activity.

The difference in the percentages of negative clones from the two biopsy specimens may be related to the small number of clones sampled. However, quantitative analyses of the uncloned specimens suggested that we might find such a difference between the two. Bi-

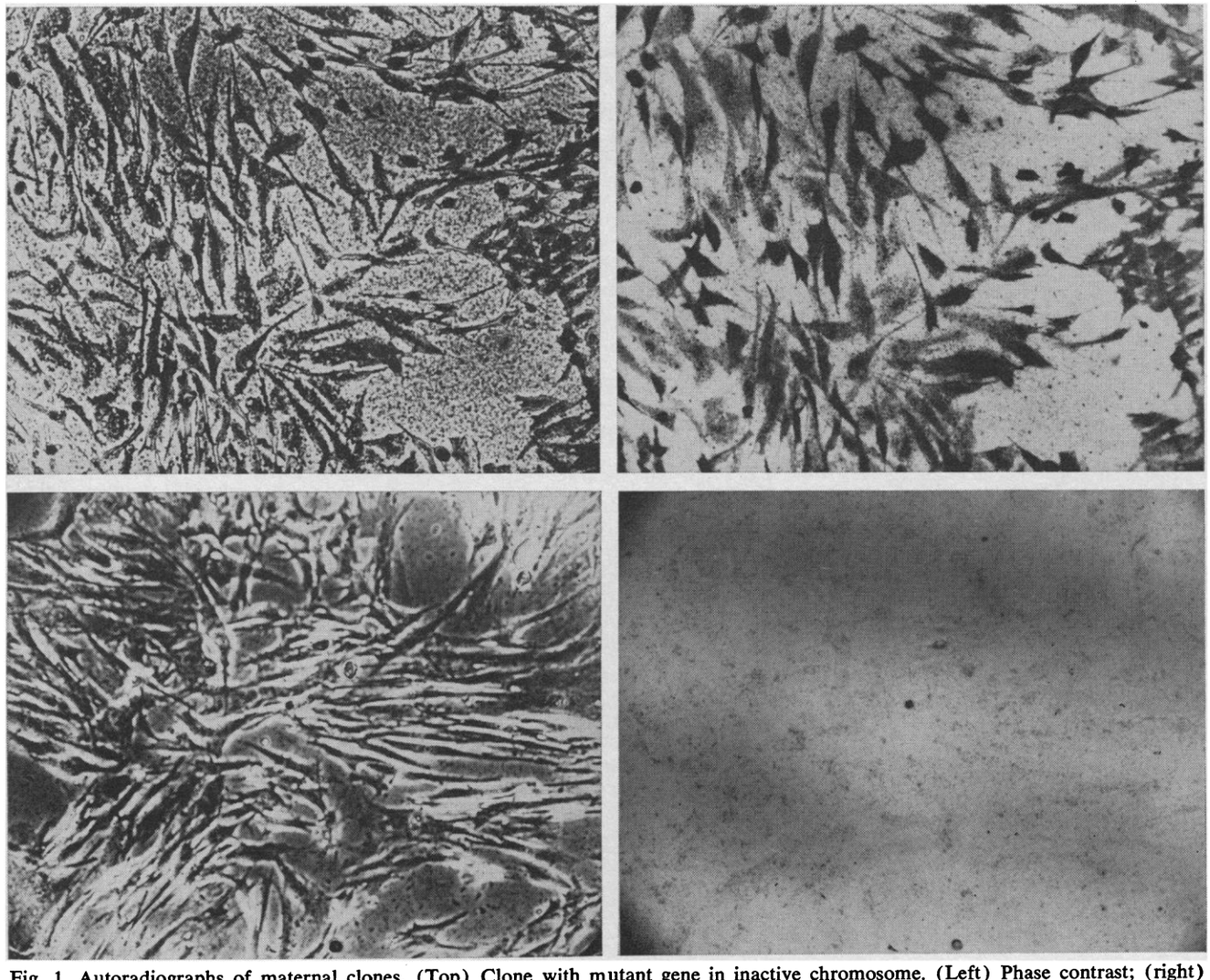

Fig. 1. Autoradiographs of maternal clones. (Top) Clone with mutant gene in inactive chromosome. (Left) Phase contrast; (right) bright field. (Bottom) Clone with mutant gene in active chromosome. (Left) Phase contrast; (right) bright field. The ^{3}H-guanine incorporated into guanylic acid appears as black granules outlining the cells. Cells with the mutant gene in the active chromosome are visible only with phase contrast (× 130).

opsy No. 2 consistently had higher amounts of radioactivity and was the source of more wild-type clones than biopsy No. 1. Although we find that both methods easily distinguish mutant from wild-type cells, they are not refined enough to permit us to interpret the range of values for HGPRT activity.

If random inactivation of loci on the X chromosome in somatic cells of the female has occurred early in development, one would expect to demonstrate two distinct cell phenotypes for X-linked loci in the heterozygote (*5*). Davidson *et al.* (*8*) and DeMars and Nance (*9*) showed two populations of cells in clones from heterozygotes having glucose-6-phosphate dehydrogenases with different electrophoretic mobilities. Danes and Bearn have shown that fibroblasts cloned from females heterozygous for the X-linked recessive form of Hurler's syndrome also fall into two populations, those with increased cellular uronic acid and metachromasia and those with normal uronic acid and tinctorial response (*10*). The HGPRT locus is thus the third for which clones of skin fibroblasts from a female heterozygote show two distinct cell phenotypes.

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Dimethyl Sulfoxide: Interactions with Aromatic Hydrocarbons

Abstract. *Dimethyl sulfoxide (DMSO) enhanced the hypertaurinuria produced by benzene, chlorobenzene, and toluene in rats. Undiluted DMSO was more effective than DMSO diluted with water in potentiating the toxicity of benzene in both rats and mice. Supernatants (9000g) prepared from livers of rats treated with DMSO 24 hours earlier metabolized more benzene than those from control rats.*

The introduction of dimethyl sulfoxide (DMSO) into clinical medicine was accompanied by reports emphasizing its relatively low toxicity and its apparent lack of interaction with a variety of drugs (*1*). Later studies have shown, however, that parenterally administered DMSO may have a greater potential for producing systemic effects than originally suspected (*2*). Of interest is the recent observation that DMSO increased the hepatotoxicity and the lethal effects of carbon tetrachloride (*3*). The studies reported here show that DMSO markedly potentiated the toxicity of several aromatic hydrocarbons and that liver preparations from DMSO-treated rats show an increased capacity to metabolize benzene. These observations suggest that caution is necessary in considering DMSO as simply an inert solvent unlikely to interact with other agents or to produce toxic effects itself.

Although taurine is primarily an intracellular compound, significant quantities are normally found in urine (*4*). Since increased levels of urinary taurine are seen after whole body x-irradiation, toxic doses of colchicine (*5*), or carbon tetrachloride (*6*), hypertaurinuria can be considered an early sign of toxicity. In the experiments reported here taurine excretion was not increased in controls treated with either saline or DMSO alone. After the injection of benzene, chlorobenzene, or toluene without dilution into the peritoneal cavity of rats, urinary taurine excretion increased markedly over the levels seen during the preceding 24-hour control periods (Table 1); however, these doses of the aromatic hydrocarbons produced no deaths. When DMSO, either undiluted or as a 25 percent solution, was injected shortly before the aromatic hydrocarbons, taurine excretion was significantly greater in these rats than in control animals given the aromatic hydrocarbons without DMSO. Furthermore, in rats pretreated with DMSO these doses of aromatic hydrocarbons did produce deaths occasionally, especially when DMSO was given without dilution immediately before the aromatic hydrocarbon.

To study the effect of diluting DMSO on the toxicity of aromatic hydrocarbons, DMSO (5 ml/kg) was given intraperitoneally to rats, either without dilution or as a 25 percent (by volume) solution in water, immediately before benzene (1 ml/kg) was administered by the same route. Mortality was compared with that observed in controls pretreated with saline. Pretreatment with undiluted DMSO produced 100 percent mortality following benzene administration to eight rats, whereas the same dose of DMSO given as a 25 percent solution produced only 11 percent mortality in 18 rats. This dose of benzene (1 ml/kg) did not kill any of 20 rats pretreated with saline, nor does this dose of DMSO (5 ml/kg) given alone produce any deaths.

The observation that closely spaced but separate injections of undiluted DMSO and benzene produced 100 percent mortality led us to explore the toxicity of benzene dissolved in undiluted DMSO. In mice the LD_{50} of benzene administered intraperitoneally with-

Table 1. Enhancement by DMSO of the urinary taurine excretion produced by aromatic hydrocarbons in rats (Wistar strain). Taurine was determined by a method previously described (*14*). Increase in the amount of urinary taurine excreted in the second 24-hour period over the control 24-hour period is expressed as micromoles of taurine excreted per rat, each rat serving as its own control. Taurine excretions in the control 24-hour periods averaged 44.1 micromoles per rat. All injections were given intraperitoneally.

Pretreatment	Treatment	No. of rats	Increase in urinary taurine excreted in 24-hour period after treatment (mean ± S.D.)
Control	Benzene, 0.5 ml/kg	11	37.7 ± 28.3
DMSO	Benzene, 0.5 ml/kg	3	93.7 ± 28.5*
Control	Chlorobenzene, 0.3 ml/kg	3	67.6 ± 4.0
DMSO	Chlorobenzene, 0.3 ml/kg	4	121.9 ± 30.8*
Control	Toluene, 0.5 ml/kg	2	36.9 ± 26.8
DMSO	Toluene, 0.5 ml/kg	2	157.7 ± 9.7*

* Significantly greater than control excretion at 0.03 level of probability.