both mutant and wild state, it seems to us likely that the relation between the population biology of a gene and the molecular biology of a gene can be established in detail.

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References and Notes

1. M. Demerec, *Genetics* **22**, 469 (1937); B. McClintock, *Cold Spring Harbor Symp. Quant. Biol.* **16**, 13 (1951); H. P. Treffers, V. Spinelli, N. O. Belser, *Proc. Nat. Acad. Sci. U.S.* **40**, 1064 (1954); T. Miyake, *Genetics* **45**, 11 (1960); E. C. Siegel and V. Bryson, *J. Bacteriol.* **94**, 38 (1967); H. Böhme, *Biochem. Biophys. Res. Commun.* **28**, 191 (1967); J. F. Speyer, *ibid.* **21**, 6 (1965).
2. C. Yanofsky, E. C. Cox, V. Horn, *Proc. Nat. Acad. Sci. U.S.* **55**, 274 (1966).
3. E. C. Cox and C. Yanofsky, *ibid.* **58**, 1895 (1967).
4. H. J. Vogel and D. M. Bonner, *J. Biol. Chem.* **218**, 97 (1956).
5. J. Monod, *Ann. Inst. Pasteur* **79**, 390 (1950).
6. T. C. Gibson and E. C. Cox, in preparation.
7. B. M. Ohlsson, P. F. Strigini, J. R. Beckwith, *J. Mol. Biol.* **36**, 209 (1968).
8. E. C. Cox and C. Yanofsky, *J. Bacteriol.* **100**, 390 (1969).
9. For a general discussion of these relationships, see A. J. Lotka, *Elements of Mathematical Biology* (Dover, New York, 1956).
10. M. Kimura, *Nature* **217**, 624 (1968); J. L. King and T. H. Jukes, *Science* **164**, 788 (1969).
11. E. C. Cox, *J. Mol. Biol.* **50**, 129 (1970).
12. Supported by NSF and by an NSF undergraduate research training grant awarded to the Biology Department.

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Lesch-Nyhan Syndrome: Preventive Control by Prenatal Diagnosis

Abstract. *The Lesch-Nyhan syndrome was detected in a fetus at a time sufficiently early to allow termination of the pregnancy. The feasibility of a preventive program for control of a severe sex-linked neurological disease through prenatal diagnosis is thus demonstrated.*

Children with the Lesch-Nyhan syndrome (X-linked uric aciduria) (*1*) have a syndrome consisting of mental retardation, choreoathetosis, muscle spasticity, and a compulsive tendency to self-mutilation, whereby the lips and distal finger are bitten away. The syndrome is inherited as a sex-linked recessive trait. Such children also show excessive purine synthesis and usually die before puberty. Since the description of the disease in 1964, approximately 150 cases of this condition have been detected, and it is likely that more remain to be discovered (*2*). No treatment is available for the neurological aspects of this severe disease. Adenine therapy (*3*) and a combined regimen of folic acid and adenine (*4*) have failed to prevent the development of the neurological features in affected children. The demonstration of a gross deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT; E.C. 2.4.2.8) in the erythrocytes and fibroblasts of affected children (*5*) has permitted the early prenatal detection of a heterozygote female fetus (*6*) and of affected twin male fetuses (*7*) late in pregnancy, which suggests the feasibility of a program for the preventive control of this disease by termination of pregnancies carrying an affected fetus. So far, however, there has been no report of detection of an affected male child at a time sufficiently early in pregnancy to allow termination of the pregnancy.

We now report the prenatal detection of a fetus with the Lesch-Nyhan syndrome before the 22nd week of pregnancy, the successful termination of the pregnancy, and confirmation of the enzyme defect in the aborted fetus. The mother had previously given birth to a normal male child and in her second pregnancy to an affected child. Radioautography performed on her fibroblasts with [^{3}H]hypoxanthine had shown two populations of cells, one with HGPRT and one without, as would be expected for the carrier of a sex-linked recessive trait by random inactivation of one X chromosome at an early stage of somatic development proposed in the Lyon hypothesis (*8*). Amniotic fluid (approximately 20 ml) was obtained in the 18th week of her third pregnancy without incident by transabdominal amniocentesis. A portion of the cells was examined for heterochromatin bodies. In addition, cells were cultured in 40 percent fetal calf serum and a mixture of medium 199 and medium NCTC 109 (1 : 1) (*9*) for radioautographic studies of their ability to fix [^{3}H]hypoxanthine. Accumulation of the radioactive label requires the presence of HGPRT, while lack of accumulation of radioactivity in the cells reflects absence of the enzyme (*6*).

Examination of the amniotic cells failed to disclose cells that contained sex chromatin, an indication that the fetus was male and that it had a 50 percent chance of expressing the disease. This was confirmed by the consistent recovery of male karyotype in amniotic cells cultured for 2 weeks. After the cells were grown in culture for 3 to 4 weeks they were subjected to radioautography with [^{3}H]hypoxanthine (*6*). Cul-

Table 1. Activity of hypoxanthine-guanine and adenine phosphoribosyltransferases in a fetus with the Lesch-Nyhan syndrome and in two other fetuses aborted for other reasons. Tissues were homogenized at 0°C in 0.05*M* tris buffer, *p*H 7.4, and 0.005*M* $MgCl_2$. Homogenates were dialyzed for 2 hours in the cold against 200 volumes of 0.01*M* tris buffer, *p*H 7.4, containing 0.005*M* $MgCl_2$ with a change of buffer at 1 hour. Assay tubes contained 50 μl of sample; 10 μl of 5-phosphoribosyl 1-pyrophosphate (PRPP), 9 m*M*; 10 μl of 0.4*M* tris buffer, *p*H 7.4, which was 0.04*M* with respect to $MgCl_2$; either 15 μl of [8-^{14}C]hypoxanthine (3.7 m*M*, 4 mc/mmole) or 15 μl of [8-^{14}C]adenine (1.9 m*M*; 17.3 mc/mmole); and 15 μl of water. Incubation was at 37°C for 20 minutes. Reactions were stopped by the addition of 10 μl of cold 42 percent perchloric acid with immersion in an ice bath. The tubes were brought to neutrality by addition of 10 μl of 0.5*M* tris buffer, *p*H 7.0, and 10 μl of 4.4*M* potassium hydroxide. Assay tubes were then centrifuged at 4000 rev/min for 10 minutes, and 3 μl of the supernatant was applied to a thin-layer chromatography cellulose sheet previously treated with 12 μg of the appropriate purine carriers (inosinic acid, inosine, and hypoxanthine for the HGPRT enzyme assay). The plates were developed for 10 minutes in 1.6*M* LiCl. The nucleotide and nucleoside spots were identified under ultraviolet light and cut out; the radioactivity was counted in toluene phosphor with 160 ml of Liquifluor per liter. Protein was determined by the method of Lowry *et al.* (*14*). The lower limit of detection of the assay was: the conversion of 0.3 nmole of [^{14}C]hypoxanthine to [^{14}C]inosine acid and the conversion of 0.08 nmole of [^{14}C]adenine to [^{14}C]adenylic acid. A control tube containing 10 μl of 0.1*M* EDTA instead of PRPP was run for each assay. Subtraction of the sum of the radioactivity in inosinic acid and inosine in this tube from the sum of the radioactivity in inosinic acid and inosine in the test tube gave the PRPP-dependent activity. All assays were performed in duplicate. HGPRT and APRT activities are expressed as the number of nanomoles of substrate converted per milligram of protein per hour at 37°C.

Tissue	HGPRT activity after delivery			APRT activity		
	Lesch-Nyhan	Normal		Lesch-Nyhan	Normal	
	34 hr	24 hr	48 hr	34 hr	24 hr	48 hr
Basal ganglia	< 0.3	59.7	30.6	9.0	1.2	< 0.08
Cortex	< 0.3	83.5	40.1	1.0	1.2	< 0.08
Cerebellum	< 0.3	44.2	41.3	2.6	1.4	< 0.08
Testis	< 0.3	67.0	24.0	1.6	1.2	< 0.08
Blood	< 0.3	11.2		11.2	1.4	
Skin	< 0.3	40.0	23.9	14.6	1.6	< 0.08

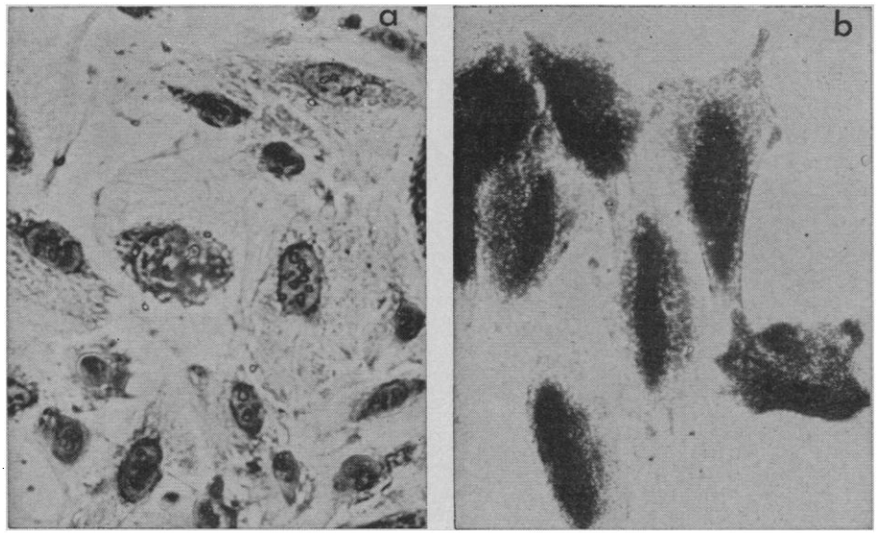

Fig. 1. Radioautography of cultured amniotic fluid cells obtained (a) from the affected fetus of 18 gestational weeks by amniocentesis, showing no accumulation of label. (b) Radioautogram of normal human amniotic cells showing dense labeling by silver granules. Both cultures were incubated with [^{3}H]hypoxanthine (24 c/mmole at a concentration of 10 mc/ml of medium for 24 hours), exposed to emulsion for 5 days, then developed and stained with Giemsa (× 200).

tures of normal human amniotic cells and HGPRT-deficient fibroblasts were used as positive and negative controls, respectively. Amniotic cells from the fetus at risk showed no appreciable uptake of [^{3}H]hypoxanthine in three separate determinations. All cells in the normal amniotic culture accumulated dense silver granules, while the HGPRT-deficient fibroblasts failed to show any appreciable accumulation of the label and were in this respect identical to the amniotic cells under study (Fig. 1). On the basis of these data obtained at the 21st week of pregnancy, the parents decided to have the pregnancy terminated. The pregnancy was interrupted by the intrauterine injection of 175 ml of 10 percent saline, and the patient delivered a 435-g stillborn fetus 24 hours later. The fetus appeared fairly well preserved, and was immediately refrigerated and subsequently shipped by air to our laboratory from the East Coast and was available for biochemical examination approximately 34 hours after abortion. At about the same time a male fetus of 22 weeks' gestation was also aborted in the same manner, and this fetus, shipped from the same hospital, served as a control and was studied simultaneously. This fetus was delivered 32 hours after intrauterine injection of 10 percent saline and consequently showed substantially more maceration than did the fetus at risk. A second unaffected fetus was obtained after saline-induced abortion, locally, and was stored for 48 hours at 4°C. The activities of HGPRT and adenine phosphoribosyltransferase (APRT; E.C. 2.4.2.7), the latter serving as control enzyme, were studied in the skin, cortex, cerebellum, basal ganglia, testis, and blood. Enzyme activities are shown in Table 1.

Hypoxanthine-guanine phosphoribosyltransferase activity was not detected in the fetus diagnosed by amniocentesis as having the Lesch-Nyhan syndrome in any of the tissues studied. In contrast, enzyme activity was present in the other two unaffected fetuses. The values recorded are lower than those recorded in adult autopsy material, and this may reflect either a true difference between fetus and adult or greater post-mortem autolysis in the fetus. Adenine phosphoribosyltransferase activity was very low in the normal fetus; the values were higher in the Lesch-Nyhan tissues, in some instances considerably so (Table 1). Activity of APRT is known to be higher in erythrocytes of affected children (*10*), but valid comparison cannot be made between the saline-aborted embryos in that the normal fetuses were more extensively macerated than the affected fetus.

The demonstration for the first time of the feasibility of prenatal detection of the Lesch-Nyhan syndrome at a time sufficiently early in pregnancy to allow its termination shows that preventive control over the disease is now practical. Index cases can be identified in cerebral palsy clinics or mental hospitals through the use of a screening procedure involving the automated measurement of the ratio between uric acid and creatinine in morning urine (*11*). When the ratio is high, the diagnosis can be confirmed by a sensitive assay for HGPRT in blood samples collected onto filter paper disks from a finger or heel puncture (*12*). Thus the diagnosis of affected children is of practical rather than theoretical importance in that this information positively identifies the female heterozygotic mothers who are at risk of bearing further affected children and raises the need for examination of other female relatives for evidence of cell mosaicism for the HGPRT enzyme. This disease may now be added to the list of disorders detectable by prenatal diagnosis (*13*).

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References and Notes

1. M. Lesch and W. L. Nyhan, *Amer. J. Med.* **36**, 516 (1969).
2. W. L. Nyhan and J. E. Seegmiller, unpublished observations.
3. J. D. Schulman, M. L. Greene, W. Y. Fujimoto, J. E. Seegmiller, *Pediat. Res.*, in press.
4. P. Benke, personal communication.
5. J. E. Seegmiller, F. M. Rosenbloom, W. N. Kelley, *Science* **155**, 1682 (1967).
6. W. Y. Fujimoto, J. E. Seegmiller, B. W. Uhlendorf, C. B. Jacobsen, *Lancet* **1968-II**, 511 (1968).
7. R. DeMars, G. Sarto, J. S. Felix, P. Benke, *Science* **164**, 1303 (1969).
8. M. F. Lyon, *Nature* **190**, 372 (1961).
9. J. A. Boyle and J. E. Seegmiller, *Methods Enzymol.* **18**, in press.
10. M. L. Greene, J. A. Boyle, J. E. Seegmiller, *Science* **167**, 887 (1970).
11. J. M. Kaufman, M. L. Greene, J. E. Seegmiller, *J. Pediat.* **73**, 583 (1968).
12. W. Y. Fujimoto, M. L. Greene, J. E. Seegmiller, *ibid.*, p. 920.
13. H. L. Nadler, A. B. Gerbie, *New Engl. J. Med.* **282**, 596 (1970).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
15. Supported in part by NIH grant AM 13622-01, by a grant from the National Foundation for Genetic and Neuromuscular Diseases, and the Tay-Sachs Association of Maryland. J.A.B. is a Harkness Fellow of the Commonwealth Fund of New York. K.O.R. is a postdoctoral fellow of the Arthritis Foundation. K.H.A. is supported by an NIH predoctoral fellowship.

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