each cell and to their close constant physical proximity, to that same extent these relations are absent in this cancer that kills with uniform regularity.

There is increasing awareness that understanding of cell reproduction entails consideration of more than the molecular particulars of chromosomes and their sequelae (*8*), and the centriole more than any other cell organelle must be included in such a consideration (*9*). Compared to the highly ordered reproduction that characterizes normal cells, malignant tumor cells reproduce as if they had been released from a critical regulatory restraint (*10*). The observations reported here may be interpreted in widely varying ways, but inescapable will be the need to explain the pattern of long-range physical order that has now been observed among the centrioles of both normal and malignant cells. If one abjures vitalism, much will be found to commend a hypothesis that centrioles interact electromagnetically; indeed, such a centriolar function may have much to do with that inexorable continuum that has characterized the diverse evolution of cellular life. Human esophageal cancer clearly represents a discontinuity in that continuum, and the data presented here strongly suggest the presence of a defect in integrity of the centriolar angle.

PAUL W. SCHAFER
Veterans Administration Hospital, Washington, D.C. 20422

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24 March 1969

Lesch-Nyhan Mutation: Prenatal Detection with Amniotic Fluid Cells

Abstract. *Cells cultured from the amniotic fluid of a 22-week fetus in a heterozygote for the X-linked Lesch-Nyhan mutation, which results in neurological and developmental disorders, lacked sex chromatin and were unable to incorporate hypoxanthine. The diagnosis of a mutant male was confirmed upon birth of enzyme-deficient, hyperuricemic twin boys whose amniotic membrane cells failed to incorporate hypoxanthine.*

We report here the prenatal detection of the inborn error of metabolism that is clinically expressed after birth as the Lesch-Nyhan syndrome (*1*). Amniocentesis ("amnion puncture") was used to sample embryonic cells in the amniotic fluid in order to determine the fetal genotype (*2*). The disease, which involves severe developmental and neurological disorders, as well as overproduction of uric acid, occurs in males having a recessive, X-linked mutant gene determining extreme deficiency in hypoxanthine-guanine phosphoribosyltransferase (E.C. 2.4.2.8) (*3*). Autoradiography distinguishes between deficient and nondeficient cultured cells; mutants do not incorporate radioactive hypoxanthine from their medium and appear unlabeled (*4*). Heterozygous females, who are unaffected, can also best be identified with autoradiography. Where a woman has had at least one affected son and there is a family history of the disease, skin biopsies have yielded the two phenotypic classes of cells expected if the gene behaves according to the Lyon (*5*) or "single-active-X" (*6*) hypothesis: "mutant" (that is, unlabeled), where the normal allele was on the inactive X, and "normal," where the normal allele was on the active X chromosome (*4, 7, 8*).

Amniotic fluid (about 10 ml) was obtained from a presumptively heterozygous woman [No. 259, in (*8*)] by transabdominal amniocentesis (*9*) during week 22 of pregnancy. Two kinds of observations were attempted with amniotic fluid cells before and after they were cultured (*10*) in vitro: (i) microscopic examination of Feulgen-stained nuclei (*11*) for the presence of Barr, sex-chromatin bodies (*12*) (absence of this indicator of the presence of two X chromosomes per cell suggests that the fetus is male); and (ii) autoradiography (*8*) was performed in order to determine whether the cells could incorporate radioactive hypoxanthine. Together, the two kinds of observations permit distinction between the four probable types of fetuses (*2*).

Most nuclei of uncultivated cells were wrinkled or had clumped chromatin and were unsuitable for scoring sex chromatin. Barr bodies were absent in 100 satisfactory nuclei, thus suggesting that the fetus was male. Too few uncultured cells remained after autoradiography to permit reliable conclusions about their ability to incorporate tritiated hypoxanthine.

The experimental amniotic fluid cells (strain No. A28) failed to grow appreciably for 26 days in Eagle's medium (*13*) supplemented with fetal bovine serum (15 percent) under conditions used for growing such cells from 16 previously sampled fetuses. Cells obtained from a normal, 18-week fetus did grow under these conditions and yielded control strain A26, which was cultured and tested in parallel with strain A28. Our concurrent studies with cultured, nonembryonic, skin fibroblasts indicated that mutant cells grew poorly unless supplied with exogenous adenine or with much higher concentrations of folic acid than are required by normal cells (*14*). While Eagle's medium does supply enough folic acid for mutants, we decided to try medium F10 (*15*): it contains enough folate and, in addition, other factors which promote superior growth of both mutant and normal fibroblasts. Replacement of Eagle's medium with F10 supplemented with 15 percent fetal bovine serum resulted in rapid growth of both cultures and permitted subculture of strain A28 onto cover slips at day 37. Strain A26 cells were fibroblastic (Fig. 1A), while A28 cells were epithelioid (Fig. 1B). The nuclei of both strains lacked Barr bodies (Fig. 1, C and D), and both fetuses were presumed to be male.

The cells of both strains incorporated tritiated adenine (Fig. 2, A and C), an indication that their machinery for synthesizing nucleic acids was intact.

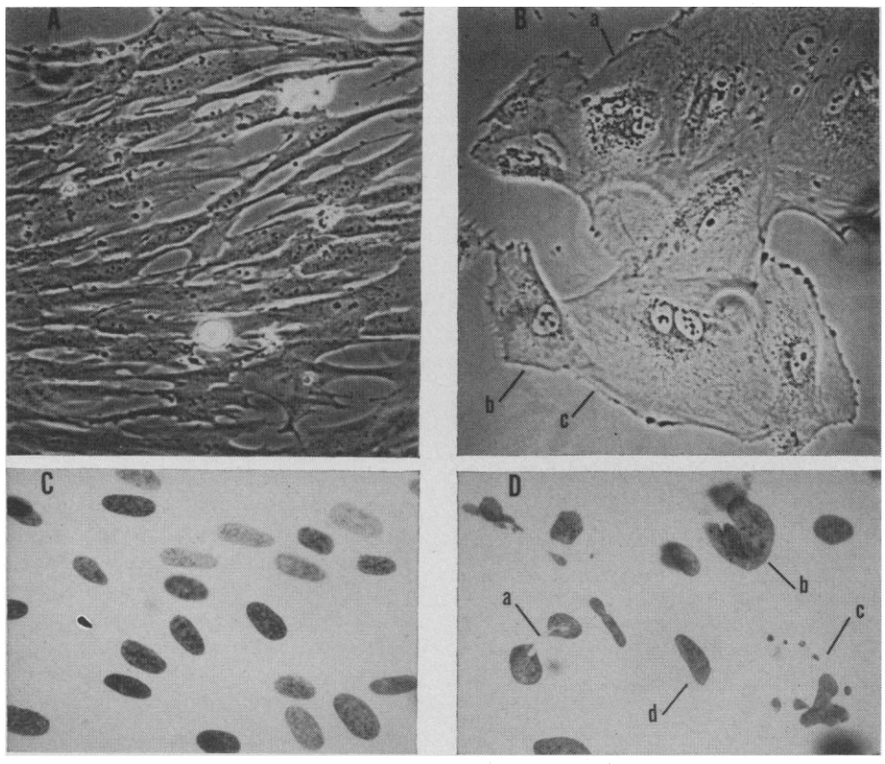

Fig. 1. (A) Phase-contrast (Zeiss optical system) appearance of living, fibroblastic cells (strain A26) cultured from the amniotic fluid of a normal, male fetus. (B) Epithelioid, amniotic fluid cells (strain A28) cultured from a male fetus with the Lesch-Nyhan mutation. Pointers indicate a cell with several micronuclei (*a*), an apparently normal cell (*b*), and a large, binucleate cell (*c*) (A and B, about × 250). (C) Feulgen-stained nuclei of cells from strain A26. The nuclear morphology is regular, and sex chromatin bodies are absent. (D) Feulgen-stained nuclei of strain A28. Pointers indicate a chromatin bridge (*a*), a giant, deformed nucleus (*b*), micronuclei (*c*), and an apparently normal nucleus (*d*) (C and D, about × 715).

Control cells also incorporated tritiated hypoxanthine (Fig. 2B) but A28 cells did not (Fig. 2D). The diagnosis of a mutant, male fetus was made during week 28 of pregnancy. It was regarded as tentative because no detection of the Lesch-Nyhan mutation prior to birth had been reported and confirmed by the birth of a mutant male.

The tentative diagnosis was confirmed by the unexpected birth of identical twin boys. Erythrocytes in their cord blood had less than 0.2 percent of the phosphoribosyltransferase activity of a control assayed (*16*) at the same time. Twelve hours after birth the uric acid concentration in their serums were 17 and 18 mg/100 ml, far above normal. Immediately after birth, numerous pieces (about 4 by 4 mm) of both amnions were placed in culture medium containing tritiated adenine or tritiated hypoxanthine. Autoradiography (*8*) showed that the cells of both amnions were labeled with adenine but not with hypoxanthine.

Fujimoto *et al.* (*2*) have reported the prenatal detection of a female fetus, which was presumed to be heterozygous for the mutant gene we studied. Both fibroblastic *and epithelioid* cells cultured from amniotic fluid formed colonies of the "mutant" and "normal" types after labeling with tritiated hypoxanthine (*2*). Nuclear abnormalities similar to those evident in strain A28 (Fig. 1, B and D) have since been observed by us in cultures of epithelioid cells derived from normal fetuses and are uncommon, in comparison, among cultured fibroblastic cells (Fig. 1C). Interpretations of metabolic and cytologic changes in such cultures (*17*) should take into account the occurrence of varying proportions of diverse cell types and possible differences between them.

Amniocentesis is used in the study of fetuses bearing a large risk of having a serious chromosome abnormality (*18*). Chromosome studies can then define the risk for a given fetus as essentially 0 or 1. This is also true for the growing list of biochemically defined errors that can be so detected (*19*) and is an improvement over the usual *statistical* risks, such as ½, ⅓, and ¼, which are assigned when the genotype of the individual fetus is not investigated. Since amniocentesis is being safely performed at the 10th week of fetal development, diagnoses can be made early enough to be an important consideration in gestational management. Such information will aid parents and physicians in defining and choosing between optional courses of action. Ameliorative treatment of the developing fetus may be elected, as exemplified by the intrauterine transfusion of Rh(+) fetuses in Rh(−) mothers. Abortion may be elected in other cases. Maximizing the opportunities for making informed decisions has proceeded swiftly in the case of the Lesch-Nyhan syndrome. Heterozygous women can be detected and informed of their chances of having affected children. Such information can be obtained about heterozygotes before they start reproducing and be made available to them when appropriate. The sex and mutant or normal status of fetuses in heterozygous women can be defined.

However, our effective use of these opportunities was impeded. (i) Pregnancy occurred before diagnosis of the mother's heterozygosity was completed. (ii) We did not learn of the pregnancy until week 21. The time needed to study the fetal cells ruled out considerations of aborting a far-advanced fetus. Nevertheless, it was important to grasp the opportunity to make prenatal diagnosis. The information gained would be applicable where amniocentesis could be performed earlier in other pregnancies at risk. The mother understood that by submitting to the procedure she would not be affecting the outcome of her own pregnancy but might ultimately be helping others. (iii) The presence of twin fetuses was missed, and it usually is about 50 percent of the time. This remarkable aspect of our study will not long remain unique as amniocentesis becomes more widely practiced. It will be necessary to make special efforts to detect twin fetuses and to insure that both amniotic fluids are sampled in cases where abortion or intrauterine treatments are possible consequences of amniocentesis diagnosis. In general, one major factor in making decisions based on genetic information is the availability of safe, effective, and economically feasible corrective measures that can be

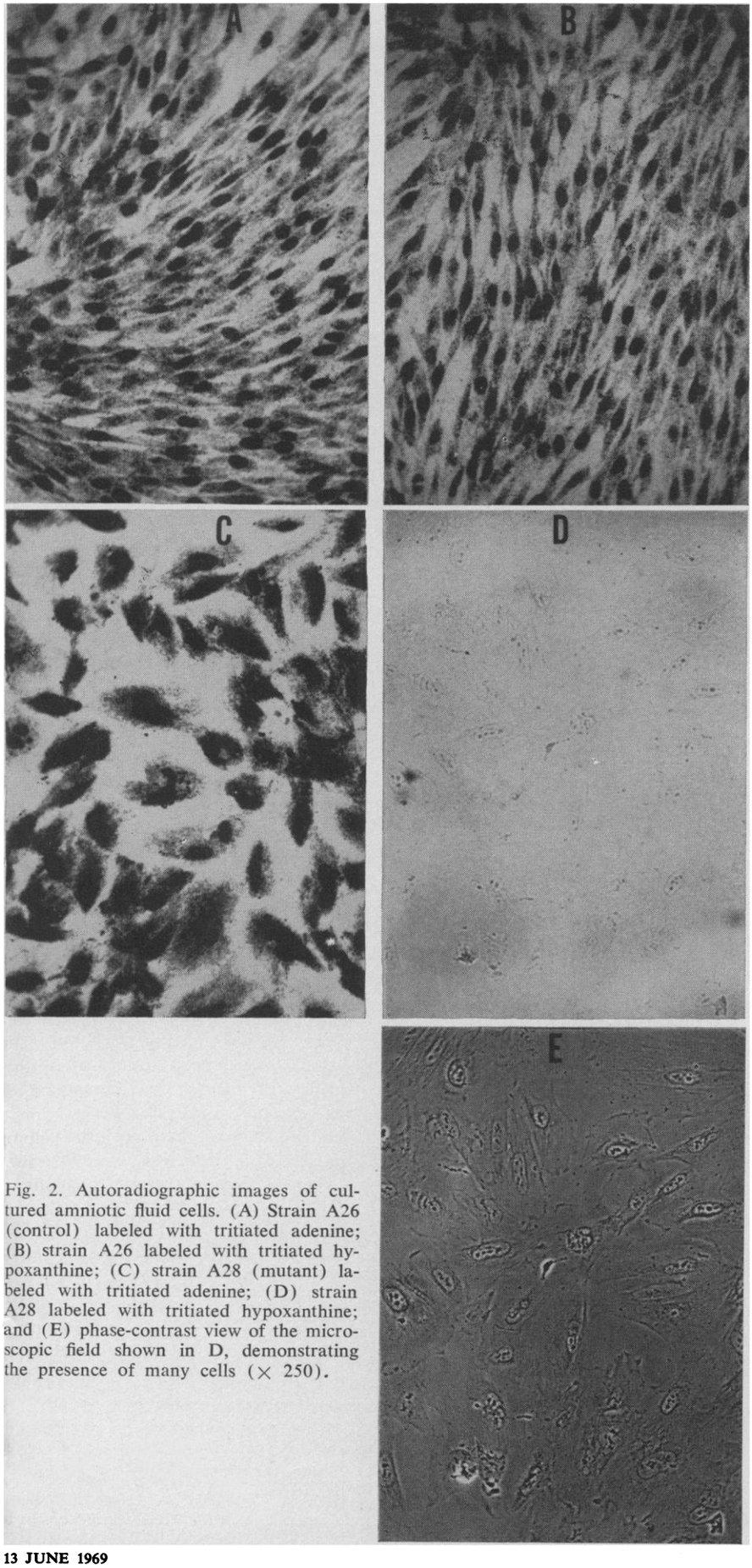

Fig. 2. Autoradiographic images of cultured amniotic fluid cells. (A) Strain A26 (control) labeled with tritiated adenine; (B) strain A26 labeled with tritiated hypoxanthine; (C) strain A28 (mutant) labeled with tritiated adenine; (D) strain A28 labeled with tritiated hypoxanthine; and (E) phase-contrast view of the microscopic field shown in D, demonstrating the presence of many cells (× 250).

applied to affected individuals. Experimental treatments for the Lesch-Nyhan syndrome that are at least partially corrective (*14, 20*) are being tested, and may even prove to be applicable to developing fetuses. Since the disease evolves gradually, it will be years before treatments can be reliably evaluated. At present, the only way in which the disease may be prevented, once conception occurs, is therapeutic abortion.

ROBERT DEMARS
Department of Medical Genetics, University of Wisconsin, Madison 53706

GLORIA SARTO
Department of Obstetrics and Gynecology, University of Wisconsin

JEANETTE S. FELIX
PAUL BENKE
Departments of Pediatrics and *Oncology, University of Wisconsin*

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21 January 1969