epoxy-2-methyloctadecane. The high specificity of the sex lure is evident from the great differences in activity encountered with slight variations in molecular structure.

BARBARA A. BIERL
MORTON BEROZA
Entomology Research Division,
U.S. Agricultural Research Service,
Beltsville, Maryland 20705
C. W. COLLIER
Plant Protection Division,
U.S. Agricultural Research Service,
Gulfport, Mississippi 39501

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7. Activity of fractions was determined by laboratory or field bioassay. In the unpublished laboratory procedure devised by one of us (C.W.C.), the sample is injected into a gas chromotograph, and the number of moths [clipped by their wings to a rack as described by B. C. Block, *J. Econ. Entomol.* **53**, 172 (1960)] activated by the effluent is compared to the number activated by an arbitrary standard. The laboratory bioassay, which registers excitement and possible attraction, was used when field testing was not possible. The field bioassay, available only a few weeks of the year, was the ultimate test of activity. Field bioassays were carried out as described in (*1*). We thank Dan Cook and Robert Tardiff of the Plant Protection Division, Otis Air Force Base, Mass., for conducting these bioassays.
8. Florisil, 60/100 mesh, activated overnight at 110°C. Fisher Scientific Co., No. F-100.
9. E. Kovats, *Advan. Chromatogr.* **1**, 229 (1966).
10. Retention times of the active material were equivalent to a C_{20} *n*-alkane on an SE-30 packing and to a C_{22} to C_{23} *n*-alkane on the more polar Carbowax 20M and DEGS packings.
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12. Adsorbosil CABN, 60/100 mesh, 25 percent $AgNO_3$ on silica gel (Applied Science Laboratories, State College, Pa.).
13. A copper column (0.9 m by 0.9 cm, outer diameter) containing 10 percent OV-17 on 70/80 mesh Anakrom ABS (Analabs, Hamden, Conn.) at 180°C was used for the first two purifications and a copper column (1.2 m by 0.45 cm, outer diameter) containing 5 percent DEGS on base-washed, 60/80 mesh Chromosorb W at 110°C for the final purification.
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15. A copper column (3.6 m by 0.6 cm, outer diameter) containing 5 percent Carbowax 20M on 60/80 mesh, acid-washed GasChrom P was used. Small peaks with t_R values the same as those of C_9 and C_{10} *n*-aldehydes were found, indicating the presence of olefin impurities differing in double-bond position. Epoxides of the olefins that would give the C_9 and C_{10} aldehydes were synthesized and were inactive in field tests (see last two compounds of Table 1). We thank R. Sarmiento of our Beltsville laboratory for helping prepare these compounds.
16. Three developments with hexane gave zones at R_F 0.32 and 0.51 for *cis*- and *trans*-9-octadecene (supplied by John E. Russell, of the Eastern Regional Research and Development, ARS, USDA, Philadelphia, Pa.), respectively. All of the natural olefin coincided with the *cis* zone.
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18. An LKB 9000 instrument equipped with a glass column (2.7 m by 0.6 cm, outer diameter) containing 2 percent SE-30 on 80/100 mesh GasChrom Q at 180°C was used. We thank G. Bagley and W. Reichel of the U.S. Department of the Interior, Patuxent, Md., and D. P. Schwartz and C. R. Brewington of the ARS, USDA, Washington, D.C., for determining these and other spectra.
19. The NMR spectra were determined on a Varian HA-100 instrument.
20. Same column as in (*15*).

20 May 1970; revised 20 July 1970

Biochemically Marked Lymphocytoid Lines: Establishment of Lesch-Nyhan Cells

Abstract. *Two lymphocytoid cell lines have been established from a patient with the Lesch-Nyhan syndrome. The cells are deficient in hypoxanthine-guanine phosphoribosyltransferase, as demonstrated by their failure to incorporate [H^3]hypoxanthine and by their inability to grow in medium in which they were nutritionally dependent upon exogenous hypoxanthine. This represents the first establishment of presumptively permanent human lymphocytoid cell lines that are deficient in a specific enzyme.*

Several hundred apparently permanent lymphocytoid cell lines have now been established from patients with various diseases (*1*) as well as from numerous healthy individuals (*2*). A few of these cell lines, derived from patients with sex chromosome aneuploidy, are chromosomally marked (*3*). However, there has been no report thus far of the establishment of biochemically marked lymphocytoid cell lines derived from patients with inborn metabolic errors. We have recently established two lymphocytoid cell lines from a patient with the Lesch-Nyhan syndrome and have found that both cell lines are deficient in hypoxanthine-guanine phosphoribosyltransferase (HGPRT) activity.

The patient was a 2-year-old white male with mental retardation, compulsive self-mutilation, and hyperuricemia. Our method of establishing cell lines has been published elsewhere (*4*). To establish the lymphocytes of this patient, 10 ml of heparinized venous blood was mixed with 0.1 ml of phytohemagglutinin (Burroughs Wellcome) and allowed to stand at 5°C for 45 minutes, after which time the leukocytes were separated off in a plasma suspension by centrifugation at 1000 rev/min for 1 minute. Approximately 3×10^6 to 5×10^6 leukocytes were divided equally and then transferred into four 30-ml plastic tissue culture flasks (Falcon), each of which contained 10 ml of RPMI (Roswell Park Memorial Institute) medium 1640 (Grand Island Biological) supplemented with 20 percent fetal calf serum (Baltimore Biological Laboratories), 100 units of penicillin G per milliliter, and 100 μg of streptomycin sulfate per milliliter. At the time of initiation of the culture, 0.5 ml of a cell lysate from the UM-3 line (University of Michigan, third lymphocytoid line) was added to three of the four culture flasks. The lysate was obtained by freezing and thawing 1×10^7 to 5×10^7 cells suspended in 2 ml of fresh medium. The UM-3 line is a recently established lymphocytoid cell line, derived from a female patient with mental retardation of undetermined etiology. The same technique was used in establishing that line as was used for the present one, except that for the UM-3 line intact cells, rather than a lysate, of the UM-1 cell line were added to the initial cultures. The karotypes of the UM-3 and UM-1 lines are normal 46,-XX, and 46,XY, respectively.

Cultures were then incubated at 37°C, 25 percent of the medium being replaced with fresh medium every 3 to 5 days. After 7 weeks of incubation, two of the three cultures to which lysates had been added began to grow very rapidly. The fourth culture, with no lysate added, failed to show any growth. The two successful cultures are now maintained as the UM-10 and UM-11 lines. Cytogenetic studies on these two cell lines revealed a normal 46,XY chromosome constitution in each. Immunodiffusion and immunoelectrophoresis on the concentrated, spent culture mediums showed that the UM-10 cells produce immunoglobulin G (IgG) and immunoglobulin M (IgM) of type K, and that the UM-11 cells produce IgG and IgM of type L. This suggested that although both lines were established from the same venous blood

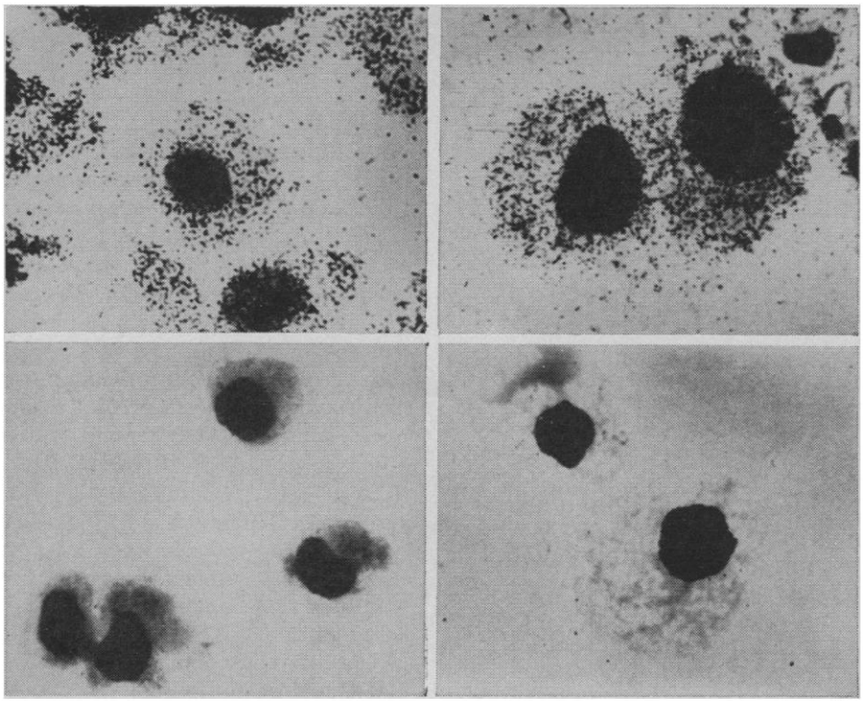

Fig. 1. Cultured normal and Lesch-Nyhan lymphocytoid cells incubated with [H^3]-hypoxanthine. (Top) Labeled cells from the normal lines, UM-1 (left) and UM-3 (right). (Bottom) Unlabeled cells from the Lesch-Nyhan lymphocyte lines, UM-10 (left) and UM-11 (right).

sample, precursor cells that differed in their kappa and lambda chain production may have been involved in the establishment of each line.

An autoradiographic study for the incorporation of [H^3]hypoxanthine was performed on the UM-10, UM-11, UM-1, and UM-3 cell lines. One million cells from each line were transferred to Leighton tubes containing 2 ml of RPMI medium 1640 with 20 percent fetal calf serum and 10 μc of [H^3]-hypoxanthine per milliliter (New England Nuclear Corporation; specific activity, 3.1 c/mmole). The cultures were incubated at 37°C for 24 hours, harvested after being washed twice with Hanks balanced salt solution, and fixed with a 1 : 3 mixture of acetic acid and methanol. The slides were dipped in the NTB^2 liquid emulsion (Eastman Kodak), exposed in the dark for 7 days, and developed in D-19 solution (Eastman Kodak).

None of the UM-10 or UM-11 cells were labeled with radioactive isotopes, while more than 95 percent of the UM-1 and UM-3 cells were heavily labeled in both the nucleus and cytoplasm (see Fig. 1). This is evidence for HGPRT deficiency in the UM-10 and UM-11 cell lines, with the block in the utilization of exogenous hypoxanthine resulting from this deficiency (*5*).

The second experiment to demonstrate HGPRT deficiency was to grow the cells in HAT medium, a selective system where the de novo synthesis of purines and pyrimidines are blocked by aminopterin, with hypoxanthine and thymidine being supplied as exogenous sources of purine and pyrimidine bases, respectively (*6*). For this experiment, the HAT medium consisted of 4 μmole of aminopterin (Lederle), 0.06 mmole of hypoxanthine, and 0.06 mmole of thymidine (Nutritional Biochemical) in 100 ml of RPMI medium 1640 with 20 percent fetal calf serum. Cells from 3-day-old cultures of UM-10, UM-11, UM-1, and UM-3 lines were suspended in fresh mediums, the cell clumps being broken by gentle agitation. Approximately 1×10^6 cells were transferred to culture dishes (35 by 10 mm), each of which contained 2 ml of either HAT medium or regular RPMI medium 1640 with 20 percent fetal calf serum. After incubation in 5 percent CO_2 for 3 days, cell growth was examined both macroscopically and microscopically.

Cells of the UM-10 and UM-11 lines, incubated in HAT medium, showed no visible growth, and degeneration of most cells was observed under the inverted, phase-contrast microscope. On the other hand, the UM-10 and UM-11 cells in regular medium and the UM-1 and UM-3 cells in both regular and HAT mediums grew actively, either singly or in clumps. Thus, the failure of the UM-10 and UM-11 cells to grow in HAT medium serves as additional evidence for the HGPRT deficiency in these cell lines.

The mechanism by which lysates of previously established lymphocytoid cell lines enhance establishment of new, presumably permanent lines is at present unclear. Nevertheless, the advantages of this technique of establishing lines are clear. First, far less blood is required for the initiation of cultures than with previously described methods (*7*). This enables us to establish cell lines from pediatric patients, including those with severe chronic illness. Further, our rate of success in establishing new cell lines by use of lysates is higher than the 20 percent success rate obtained by repeated stimulation with phytohemagglutinin (*8*). Thus far we have been able to establish 16 new cell lines from 12 of 16 hematologically normal individuals. One failure was due to bacterial contamination, and in three cases the cells started to proliferate, but the premature splitting of the cultures precluded establishment of the cell lines.

The establishment and maintenance of permanent lymphocytoid cell lines may provide an excellent alternative system to the fibroblast for the study of genetically determined biochemical defects in human cells. The lymphocytes grow readily in suspension culture and do not appear to senesce.

KYOO W. CHOI
ARTHUR D. BLOOM
Department of Human Genetics, University of Michigan Medical School, Ann Arbor 48104

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10 June 1970