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Received July 10, 1968. P.S.E.B.M., 1969, Vol. 130.

Prolonged Culture of Human Leukocytes in an Isolated Environment (33557)

WOLCOTT B. DUNHAM, W. E. VINSON, M. V. PARKER, AND I. D. CLARK

Medical Research Laboratories, Veterans Administration Hospital, Memphis, Tennessee 38104

The present study was undertaken to determine the possible role of viral or cell contamination in the long-term culture of human leukocytes from apparently normal individuals. Numerous instances have occurred of entry of cells of diverse origin into established cell lines. Therefore, apparently successful long-term cultures of hemic cells might in reality represent contamination from other cultures carried in the same laboratories. Moore *et al.* (1) suggested that the presence of herpes-like virus, such as is present in cells cultured from Burkitt's lymphoma, may be a necessary factor for continued growth of normal hemic cells.

A line of cells from human peripheral blood was established in our laboratories in an environment that precluded entry of cells from other cultures. No virus particles were revealed by an electron microscopic survey.

Materials and Methods. The research laboratories of our new hospital were available for use several weeks before the rest of the building was occupied and so provided an isolated environment for cell culture. As these conditions of isolation could not be repeated, we selected procedures for culture similar to those we used successfully in a previous study (2). New equipment and fresh supplies were employed. After growth was observed, the line was maintained in a laboratory where the atmosphere was under positive pressure and where no other cell cultures were at any time present.

Blood donors were in apparent good health. Their blood cell counts and differentials were within normal limits. They did not receive food for 10 hr before being bled. Using a vacuum system, 250 ml of blood was drawn from each of six donors and allowed to clot. The bottles were kept for 7 hr at 26° and then for 18 hr at 4°. Cells in the serum in each bottle, together with cells obtained by gently rinsing the clot, were washed twice in solution 199 (Difco). Suspensions containing about 10^5 leukocytes/ml were prepared in culture medium consisting of 80% solution 199 and 20% pooled human serum that had been heated for 30 min at 56°. Five-ml amounts of each suspension and of a pool of the six suspensions were placed in 3 × 5-cm plastic tissue culture flasks (Falcon) and incubated at 36°. Cells were fed every 3 or 4 days by replacing half the medium with fresh nutrient. When multiplication had occurred, cells were released for transfer with trypsin and washed once in solution 199.

For electron microscopy, cells were first fixed in Dalton's solution, then in 10% formalin with 0.2% uranyl acetate. Following dehydration by alcohols and propylene oxide, they were imbedded in epoxy, sectioned, and stained with lead citrate.

Results. On day 43, 7 colonies, each consisting of 8 or more cells, were observed on the culture surface of the flask that had been seeded with pooled cells (Fig. 1). By day 54 these had developed into a monolayer of cells with many mitotic figures (Fig. 2). Since then, the culture, designated KeNAHB-22 (2), has been transferred 44 times during a period of 1 year. No evidence of multiplica-

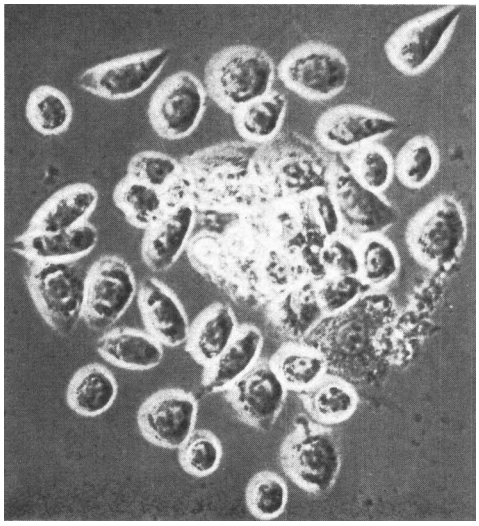

FIG. 1. Colony present after 43 days of culture; ×400.

tion was observed by day 57 in the flasks planted with cells from individual donors.

Sections of more than 1000 KeNAHB-22 cells were examined by electron microscopy. No viral particles were observed.

Chromosome analysis was made by Dr. H. Bernhardt. As in most instances of cells grown for a prolonged period on a culture surface, this line is heteroploid. A valuable contribution in this field has been made by Moore (1) who initiated hemic cultures that have remained diploid through use of the hover technique.

Cinephotomicrographic analysis demonstrated that the narrow ends of many of the pseudopods waved rapidly from side to side while exhibiting active pinocytosis. The number of pseudopods of individual cells varied from 2 to 4 within a 3-hr period. The nuclei, though varied in morphology and staining characteristics, indicated a lymphoid origin.

Discussion. In 8 of 11 instances reported, a period of more than 30 days elapsed before multiplication of cells from the peripheral blood was observed (1, 3, 4). In the present study, a lag phase of about 43 days preceded continuous and rapid growth. The large number of neutrophils present may have contributed to this delay in multiplication. For each of the cells that multiplied to produce the 7 colonies, the number of those that did not grow was originally in the order of 70,000. By the time that rapid growth occurred, almost all of these had been washed away or had disintegrated. In some instances of successful culture, transformation may occur before evidence of multiplication is obtained. However, transformation with development of polyploidy is not a prerequisite, as delayed rapid growth occurred in the hover cultures of diploid cells described by Moore (1).

Contamination of cell sublines by other cells has been reported in more than 30 instances (5). Chromosome analysis by Martin *et al.* (6) indicated that our earlier leukocyte line, KeNAHB-9, had entered concurrent HeLa cultures. The relative ease with which cell lines can become mixed and the comparative infrequency of success in establishing long-term cultures from normal human peripheral blood led Woodliff (7) to speculate that such apparent cultures may actually have been initiated by entry of cells from other lines. Our successful culture in an isolated environment indicates that cells present in the peripheral blood of normal individuals are capable of prolonged growth.

Conclusions. Cells capable of continuous propagation *in vitro* were cultured from peripheral blood of normal individuals under

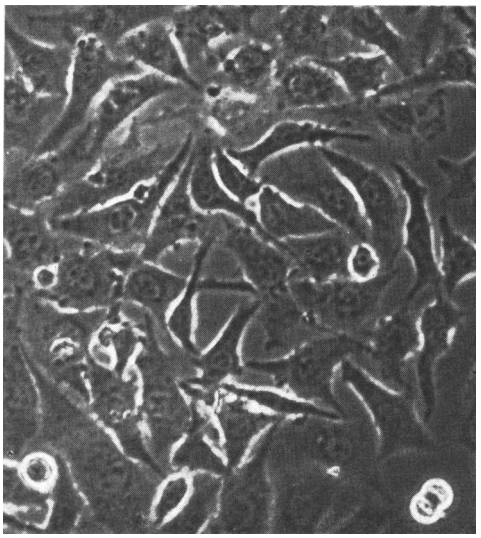

FIG. 2. Monolayer present after 54 days of culture; ×400.

conditions of complete isolation from other cell lines. A lag phase of 7 weeks preceded rapid multiplication. Cultivation of these cells is apparently not dependent on the presence of a herpes-like virus similar to the leukovirus found in Burkitt's lymphoma. The line has been cultured continuously for 1 year.

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Received Sept. 11, 1968. P.S.E.B.M., 1969, Vol. 130.

Porphobilinogen and Porphyrin Synthesis in Splenomegaly of Some Murine Leukemias* (33558)

L. A. FIELDS,[1] S. VADLAMUDI,[2] V. S. WARAVDEKAR, AND A. GOLDIN

Microbiological Associates Inc. (L.A.F., S.V., and V.S.W.) and Cancer Chemotherapy, National Service Center, National Cancer Institute (A.G.), Bethesda, Maryland 20014

Splenomegaly has been employed to measure the degree of infectivity of leukemia viruses in mice (1). Since the erythropoietic activity in mice increases after infection with murine leukemia viruses (2), the rise in Δ-aminolevulinic acid dehydratase (5-aminolevulinate hydro-lyase, E.C. 4.2.1.24) (DALAD) and porphyrin producing activities representing heme synthesis was considered to reflect the extent of infection with these viruses in the host systems. Tengerdy reported that DALAD activity increased in tissues of mice infected with Friend virus (FV) (3), and we also observed (4) increased DALAD and porphyrin activities in spleens of mice infected with FV and Rauscher virus (RV). Although splenomegalic response was similar in mice infected with these viruses, the porphyrin activity in spleens of mice infected with FV was twice the activity observed in spleens of mice infected with RV. Experiments were conducted to examine the effects of FV infection on the enzymic activity of various strains of mice and to correlate the sensitivity of the enzymic system with the degree of viral infection.

Materials and Methods. BALB/c, DBA/2, BDF_1 (C57BL/6♀ × DBA/2♂)F_1, and C57BL/6 mice 7–10 weeks of age and weighing 18–23 g, were inoculated intraperitoneally with 0.2 ml of a $10^{-1.3}$ dilution of a plasma virus preparation of FV. The DBA/2 mice were inoculated subcutaneously with 0.2 ml of 1×10^6 leukemic cells in physiologic saline prepared from spleens of DBA/2 stock leukemic (L1210) mice. Five mice from each group of normal, virus infected or L1210 inoculated animals were sacrificed by cervical dislocation at designated intervals. Spleens were removed, weighed, washed three times

* Supported by Contract PH-43-64-911 from the Cancer Chemotherapy National Service Center, National Cancer Institute, NIH, USPHS.

[1] Present address: Carter Wallace Company, Wallace Laboratory Section, Cranberry, New Jersey 08540.

[2] Reprint requests should be addressed to S. Vadlamudi, Microbiological Associates, Inc.