Growth of a Human Leukemia Cell Line on Protein-Free Medium[1] (36646)

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(Introduced by C. C. Congdon)

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Various authors (1–13) have described techniques for culturing mammalian cells on media that do not contain serum. In many cases, however, the cells have been maintained on media that were not chemically defined but contained one or more of the following: insulin (1), lactalbumin hydrolysate (2, 3), bacto-peptone (4,5), hydrolysates of human and horse serum albumin (6), or fetuin and albumin (7–9).

Mouse L cells were cultured in a defined medium by Bryant *et al.* (10) but grew very slowly, with generation times of 2.7–9 days. Merchant and Hellman (4) later adapted a substrain, Mouse L-M, to grow on 2X Eagle's basal medium with a mean generation time of 56 hr.

Long-term cultivation of mammalian cells in a protein- and lipid-free medium has recently been described by Takaoka and Katsuta (11). Some of their substrains were capable of growing in the minimum essential medium outlined by Eagle (14). Short-term cultures of human peripheral blood cells stimulated with phytohemagglutanin have also been grown in serum-free medium (12, 13). We describe here the long-term cultivation of a human leukemia cell line in a chemically defined medium and the sequence of events regulating their growth rate.

Materials and Methods. Cell line RPMI 4265 was initially derived from a chronic granulocytic leukemia patient and is normally grown in suspension on RPMI 1640 medium (15) supplemented with 20% fetal calf serum, penicillin (50 units/ml), and streptomycin (5×10^{-5} g/ml). Cultures of this cell line were centrifuged, and the cells were washed in medium without serum supplements and incubated in RPMI 1640 medium supplemented with 0.24% methyl cellulose or minimum essential medium supplemented with serine (10^{-4} *M*), asparagine (10^{-4} *M*), and 0.24% methyl cellulose, and when indicated, fetuin (0.2 mg/ml) or albumin (5 mg/ml), at 37° in a humidified atmosphere of 2% CO_2 in air. The initial cell density was $5–7 \times 10^5$ cells/ml, and the medium was changed and the cells were subcultured every 6 or 7 days.

Growth rates were determined by removing random samples of the cultures and counting the number of cells with a hemacytometer and electronically in a Coulter counter. The viability was determined by the erythrosin B dye-exclusion method (16).

The relative rates of DNA and RNA synthesis were determined by incubation of cell samples in medium with and without serum, containing 1 μCi/ml [^{3}H] thymidine (sp act, 1.9 Ci/mmole and [^{3}H]uridine (sp act, 2 Ci/mmole), respectively. Fractions were removed at various times, the cells were counted, and sonicated, and the trichloroacetic acid-insoluble radioactive fraction was counted in a BBOT-toluene scintillating solution (17). Protein synthesis was measured similarly, using 0.9 μCi/ml [^{14}C]leucine (sp act, 311 Ci/mole) incorporation as the assay.

Results. Figure 1 shows growth curves from human leukemia cell line RPMI 4265 grown in various media. Cultures of cells

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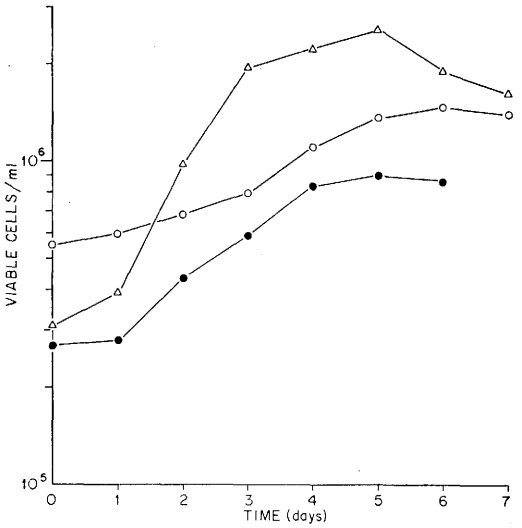

FIG. 1. Growth curves of RPMI 4265 grown in (△) RPMI 1640 plus 20% fetal calf serum (av viability, > 90%); (○) RPMI 1640 plus 0.24% methyl cellulose (av viability, 85%); and (●) minimum essential medium plus 10^{-4} *M* serine, 10^{-4} *M* asparagine, and 0.24% methyl cellulose (av viability, 80%). Cells grown without serum were grown for 3–4 weeks before growth rates were determined.

grown in serum-free medium were maintained without protein supplements 3–4 weeks before the growth rate was determined. The doubling times of the cells grown in medium supplemented with serum were 18–24 hr, whereas the doubling times of the cells grown in RPMI 1640 without serum supplement were 46–52 hr. Cells could also be grown on the minimum essential medium described by Eagle (14), when supplemented with serine and asparagine (18). These cells had doubling times similar to those of cells grown in RPMI 1640 medium without protein supplements.

The viability of the cells grown in the chemically defined medium averaged 80–85% and was lower than that of cells grown in medium containing serum, which was greater than 90%. Addition of either fetuin or albumin to the defined medium stimulated cell growth to the level observed in cells grown on medium containing serum; however, there was a lag before the rapid growth rate was achieved (Fig. 2).

Figure 3 shows the relative rates of DNA, RNA, and protein synthesis of cells grown in the presence and absence of serum. The rate of [^{3}H]thymidine uptake by the cells grown in the chemically defined medium was 40–50% of the rate for cells normally maintained in medium containing calf serum. The rate of RNA synthesis by cells grown without serum was also reduced, but only to 60% of the rate observed in the rapidly growing cells. Protein synthesis in cells grown in the chemically defined medium was reduced to 40% of the synthesis in cells grown in partially defined medium. Some interesting observations from Fig. 3 are (a) when cells normally maintained in medium containing serum are deprived of serum, DNA and RNA synthesis continue at the same rate during the first 24 hr, but protein synthesis is reduced; and (b) when cells that have been maintained in medium lacking protein supplements are incubated in medium containing serum, DNA synthesis is not stimulated during the first 24 hr, whereas RNA synthesis increases after 12 hr, and the rate of protein synthesis increases immediately.

Discussion. Human leukemia cell line RPMI 4265 can be maintained on a chemically defined protein-free medium, although the growth rate is reduced. We have main-

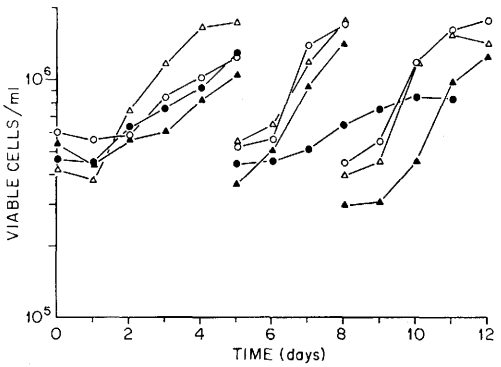

FIG. 2. Growth curves of cell line RPMI 4265 grown in (△) RPMI 1640 plus 10% calf serum; (○) RPMI 1640 plus 0.24% methyl cellulose and 5 mg/ml albumin; (▲) RPMI 1640 plus 0.24% methyl cellulose and 0.2 mg/ml fetuin; and (●) RPMI 1640 plus 0.24% methyl cellulose. Cells grown without calf serum were grown in chemically defined medium 3–4 weeks before growth curves were determined.

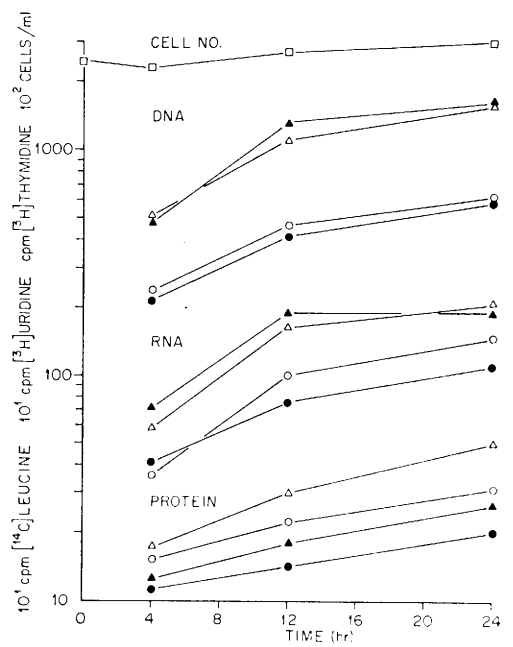

FIG. 3. Uptake of radioactive precursors for DNA, RNA, and protein in 0.1-ml samples of RPMI 4265 cultures incubated in [^{3}H]thymidine, [^{3}H]uridine, and [^{14}C]leucine under different conditions. (△) Cells maintained in RPMI 1640 medium supplemented with 10% calf serum and labeled in the same medium; (▲) cells maintained in RPMI 1640 medium supplemented with 10% calf serum and labeled in RPMI 1640 medium plus 0.24% methyl cellulose; (●) cells maintained in RPMI 1640 medium plus 0.24% methyl cellulose and labeled in the same medium; (○), cells maintained in RPMI 1640 medium plus 0.24% methyl cellulose and labeled in RPMI 1640 supplemented with 10% calf serum.

tained cultures of these cells for periods greater than 5 months on medium without proteins or serum. The viscosity of the medium, either RPMI 1640 or minimum essential medium plus serine and asparagine, can be increased by the addition of methyl cellulose which has been found to produce a protective effect by reducing cell fragility (4). We have found, however, that even in the presence of methyl cellulose care must be taken not to manipulate the cells excessively and not to disrupt the cell aggregates which normally form in this suspension cell line. If the cells are manipulated and the aggregates disrupted, the result is a lowered viability and a depression of growth rate. The growth rate is also depressed if the cell density is reduced below 3–5 $\times$ 10^5 cells/ml.

There is a correlation between the lowered growth rate of the cells on the defined medium and the reduced rates of DNA and protein synthesis in these cells, compared to the rates in cells maintained on supplemented medium; however, there is no direct correlation between the reduced growth rate and the rate of RNA synthesis. In addition, in step-up (addition of serum) and step-down (removal of serum) experiments, the rate of protein synthesis is altered first. Therefore, we conclude that an important substrate for, or regulator of, protein synthesis is present in the serum and absent from the chemically defined medium. The data also imply that cell growth is regulated through protein synthesis.

Summary. Human leukemia cell line RPMI 4265 was maintained on a chemically defined medium without protein supplements. The doubling time of the cells was 46-52 hr, whereas cells grown on medium supplemented with serum had a doubling time of 18–24 hr. The rates of DNA, RNA, and protein synthesis were also reduced. Growth could be stimulated by addition of fetuin, albumin, or serum to the medium, but there was a lag before the rapid growth level was attained.

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