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Cell Proliferation and Immunoglobulin Production in Human Hematopoietic Cell Culture

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The nutritional requirements generally satisfied by serum in established cultures of human hematopoietic cells were studied in spinner culture. The optimum concentration of fetal calf serum for cell proliferation in medium RPMI #1640 appeared to be 20%, and the minimum to be 2%. In the logarithmic phase of cell growth, there was an exponential increase of protein in the cells and a corresponding decrease in the medium. Immunoglobulin production in one cell line was highest in the logarithmic phase of cell growth and in the culture medium supplemented with serum at a low level. The rate of cell proliferation under various culture conditions bears some relationship to the rate of immunoglobulin production in these cell lines.

INTRODUCTION

A number of human hematopoietic cell lines have been originated from the buffy coat of peripheral leukocytes from patients with diverse clinical diseases, leukemia, and even from healthy individuals[1-3]. Because of their unique properties, such as immunoglobulin production[4-9] and the frequent presence of a herpes-type virus[10,11], great interest has been focused on these lines. Recent experiments in our laboratory have shown that some human hematopoietic cells grow in chemically defined media without supplementation with serum or other macromolecules.

The present study is concerned with the effects of various concentrations of serum in the medium on cell growth in suspension culture. Growth rate was correlated with the functional activity of the cells under various nutritional conditions by evaluating μ-chain reactive

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immunoglobulin production with immunofluorescence methods.

MATERIALS AND METHODS

The human lymphoblastoid cell lines used in this study were RPMI #5328, #4265, and #7206[1-3]. RPMI #5328 was derived from the peripheral blood of a patient with an acute myelogenous leukemia, #4265 from a patient with a chronic myelogenous leukemia, and #7206 from a healthy individual. The procedures for establishment and maintenance and the general characteristics of these cell lines have been reported[1,2]. The karyotypes of the cells have been diploid more than 2 years.

Medium RPMI #1640[2] supplemented with 10% (V/V) heat-inactivated fetal calf serum or adult human serum (GIBCO) was used in 50-ml spinner flasks. The cell suspension contained $3-5\times10^5$ cells per ml with a viability of 95% or more which was estimated by the dye exclusion method, and the pH was adjusted to 7.3. The flasks were incubated in a spinner box with the magnetic stirrer at 37°C without refeeding for 7 days. Cell growth and viability were determined with the use of hemocytometer by the trypan blue exclusion test.

RESULTS

Growth behavior in spinner flasks was generally similar for the three cell lines. The cells grew only in suspension, without adherence to the glass vessel, but with some clumping that could be easily eliminated by shaking.

With inocula ranging from 3 to 5×10^5 cells/ml, growth was slow in the first 24 hours (lag phase), but exponential for the next 3 days, and then showed a plateau phase, followed by deterioration. With an inoculum of more than 5×10^5 cells/ml, the lag period was absent, and the exponential phase was brief. With an initial inoculum of less than 3×10^5 cells/ml, the doubling time was prolonged, and the exponential phase extended for more than 3 days. If the inoculum was smaller than 0.5×10^5 cells/ml, the cells did not grow. It seemed desirable to use inocula ranging from 3 to 5×10^5 cells/ml for the purpose of the present study. The maximal cell populations attained were about 3×10^6 cells/ml, regardless of the initial cell population.

Effects of serum and serum substitutes. Fig. 1 and 2 show representative results for cell line RPMI #5328 with various concentrations of fetal calf or adult human serum. The various concentrations provided similar growth patterns. Growth was greater in medium supplemented with 5, 10, 20, or 40% fetal calf serum than in medium with 1 or 2% serum. Medium without serum supplementation did not support cell

growth, and did not even maintain the original viability of the cell population. The doubling time during the exponential growth period was about 25.5 hours. The cells grew faster with 2% adult human serum than with 2% fetal calf serum.

Various concentrations of bovine albumin (Pentex), lactalbumin hydrolysate (NBC), and bactopeptone (NBC) were added to medium RPMI #1640 without serum. Although some differences among the 3 cell lines

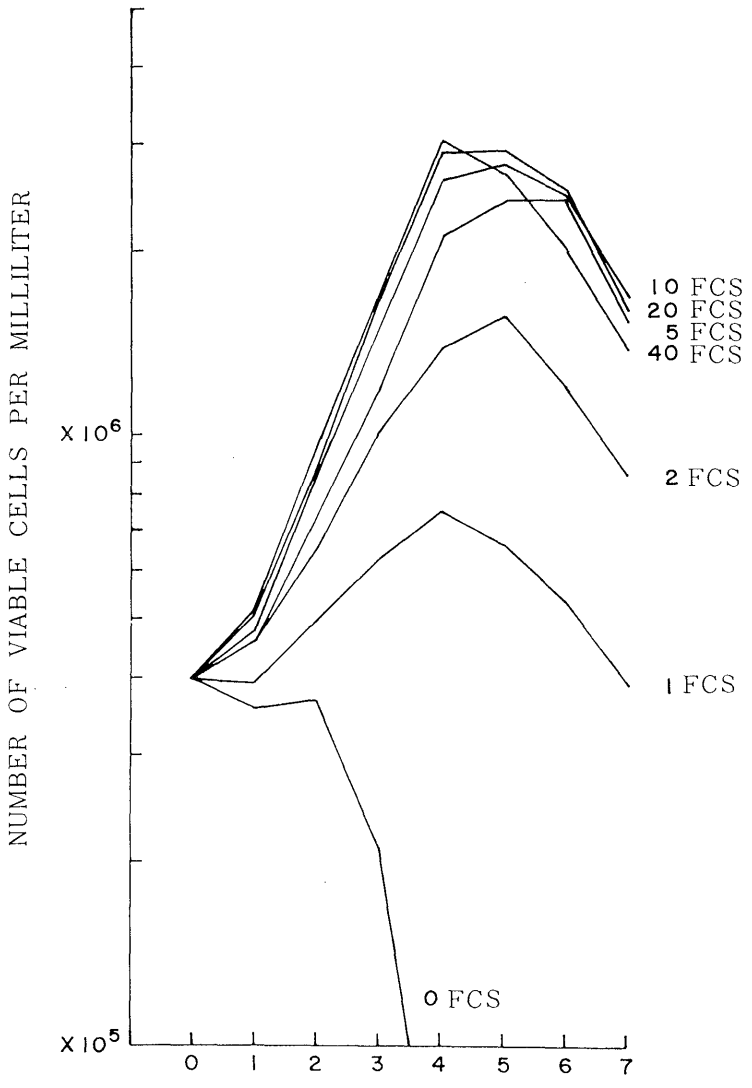


Fig. 1.

FIGS. 1 and 2. Growth curves for cell line RPMI #5328, with various concentrations of fetal calf serum (Fig. 1) and adult human serum (Fig. 2).

FCS, % fetal calf serum in medium RPMI #1640; HS, % human serum in medium RPMI #1640.

tested were found in the maximal cell population, the optimum concentrations of these supplements were 0.07% for bovine albumin, 0.2% for lactalbumin hydrolysate, and 0.1% for bactopeptone medium. These substances temporarily supported a low rate of proliferation of the cells in the absence of serum, but were not as effective as 2% serum. A combination of these supplements was not beneficially.

Medium supplemented with bovine albumin, lactalbumin hydrolysate, or bactopeptone at an optimum level contained about 700 μg/ml protein as determined by the method of Lowry et al.[12]. This protein concentration, which seemed to be optimal for cell growth, was equival-

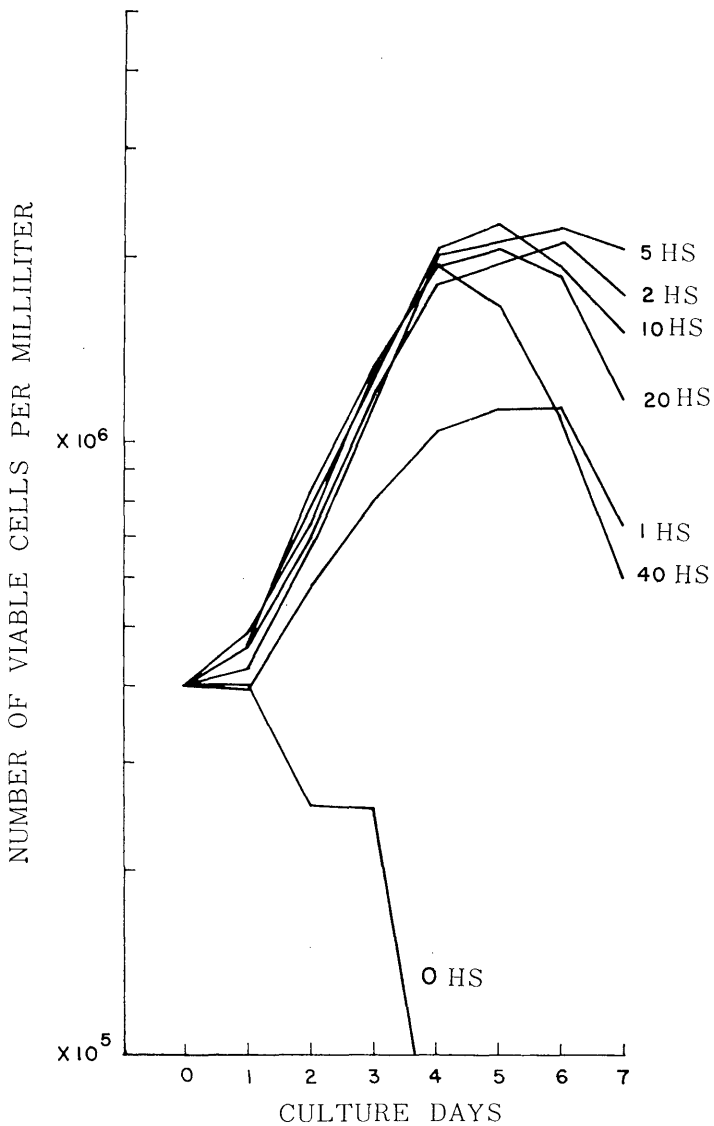


Fig. 2.

by centrifugation at 1,500 rpm for 10 minutes. The packed cells were washed 3 times with phosphate buffer solution before protein assay.

Figs. 3 and 4 show the changes in the protein contents of the cells and medium during continuous cultivation. During the period of cell proliferation, the growing cells consumed some protein from the medium, and their protein content increased. During the phase of cell

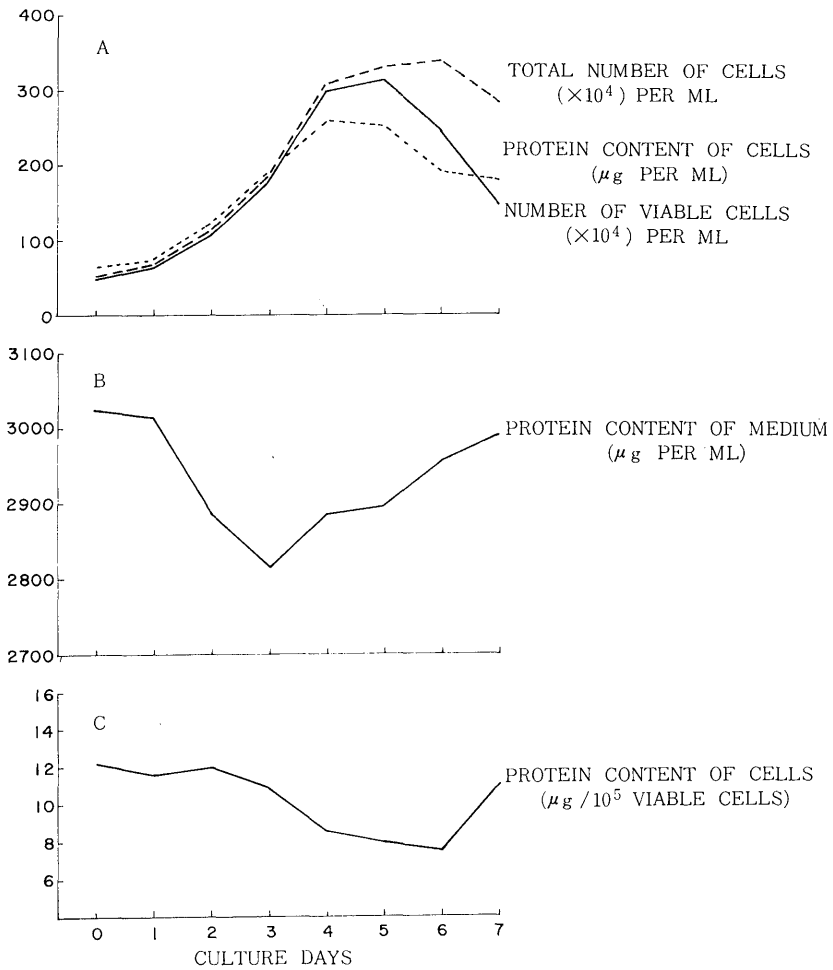


Fig. 3.

FIGS. 3 and 4. Serum protein consumption during continuous cultivation of cell line RPMI #4265 in medium containing 10% (Fig. 3) and 2% (Fig. 4) fetal calf serum.

(A) Relationship between changes in cell protein content and cell proliferation. Protein content represents the mean of 3 samples; standard deviation ±8.3 in both figures. (B) Changes in protein content in cell-free supernatant medium. Protein content represents the mean of 3 samples; standard deviation ±83.0 in Fig. 4 and ±33.2 in Fig. 5. (C) Changes in ratio of protein content to number of cells.

ent to 2% fetal calf serum or 1% adult human serum. The latter concentrations of serum appeared to be the minimums required for good cell growth, even though serum protein probably not only serves as a nutrient, but also provides osmotic support and may be a carrier of unidentified growth factors. It was interesting that little difference in effectiveness was found between 2% fetal calf serum and 1% adult human serum.

Serum protein consumption. Protein contents of cells and cell-free medium were determined by a modification of the method of Lowry et al.[12] in order to evaluate serum protein consumption by the cells. Spent medium with the cells was separated into cells and supernatant growth decline, however, the protein content of the medium increased, as might be expected as a result of protein release from degenerating cells. The ratio of the protein content of the cells to the cell population proved to be higher in the early period of logarithmic cell growth than

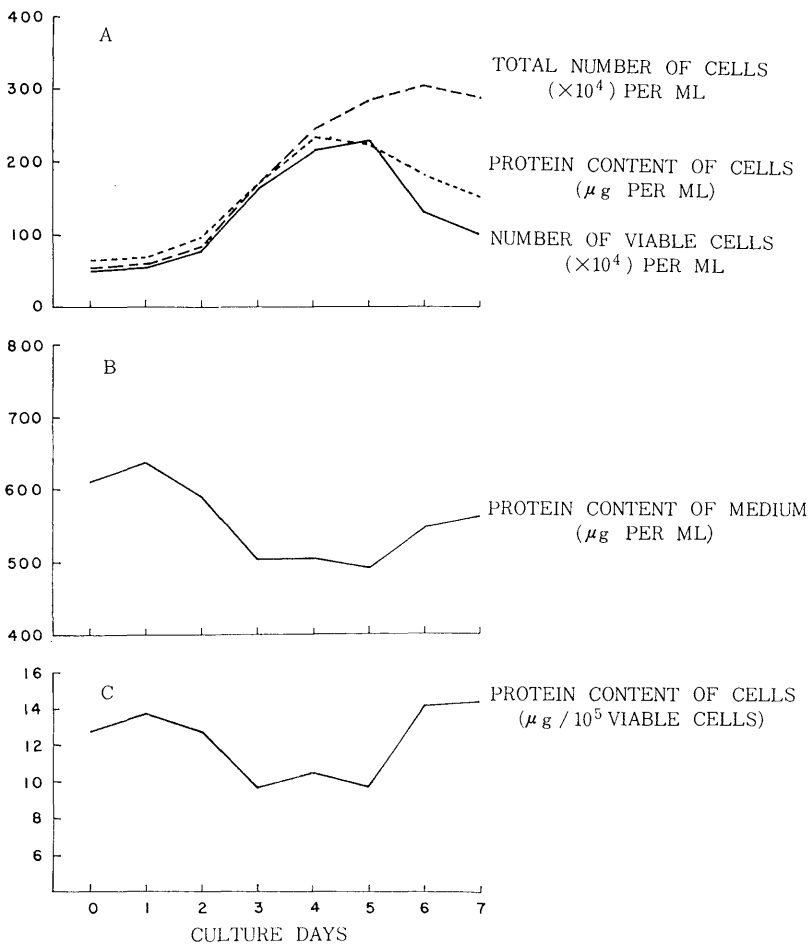


Fig. 4.

in the late period. The total protein content of the culture decreased during cell growth, but an equivalent relationship could not be found between the increased protein content of cells and the decreased protein content of the medium during cell proliferation. After 4 days of cultivation, determinations of protein in cells and medium may be inaccurate because of increasing proportions of dead cells and debris.

Cellular immunoglobulin levels. Human hematopoietic cell lines synthesize diverse classes of immunoglobulins[9]. Cell line RPMI #4265 reacted strongly with anti-human μ chain reagent (goat anti-human μ chain labeled with fluorescein isothiocyanate, Hyland Laboratories).

Figs. 5–7 show kinetic analyses of population growth, cell viability, and percentage of immunoglobulin-positive cells in medium supplemented with 10, 2, or 0% fetal calf serum. It should be kept in mind that immunofluorescence reflects the presence of reactive antigen and does not indicate active synthesis at the time of determination. The percentage of immunoglobulin-positive cells proved to be highest in the loga-

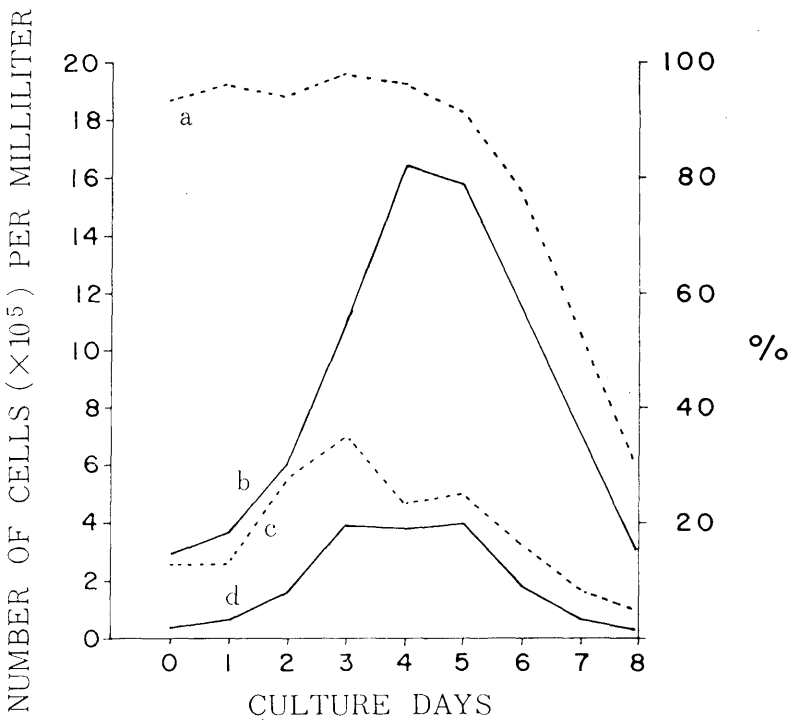


Fig. 5.

FIGS. 5–7. Relationships among changes in cell proliferation, cell viability, and number of immunoglobulin-positive cells in medium containing 10% (Fig. 5), 2% (Fig.6), and 0% (Fig. 7) fetal calf serum.

(a) Cell viability (%). (b) Number of viable cells per milliliter. (c) Percentage of immunoglobulin-positive cells among total viable cells. (d) Number of immunoglobulin-positive cells per milliliter.

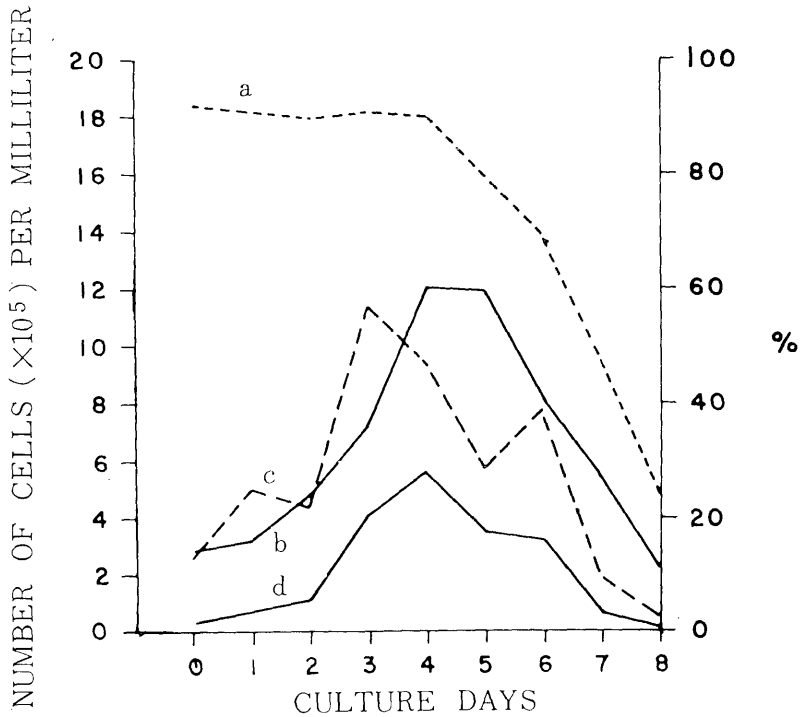
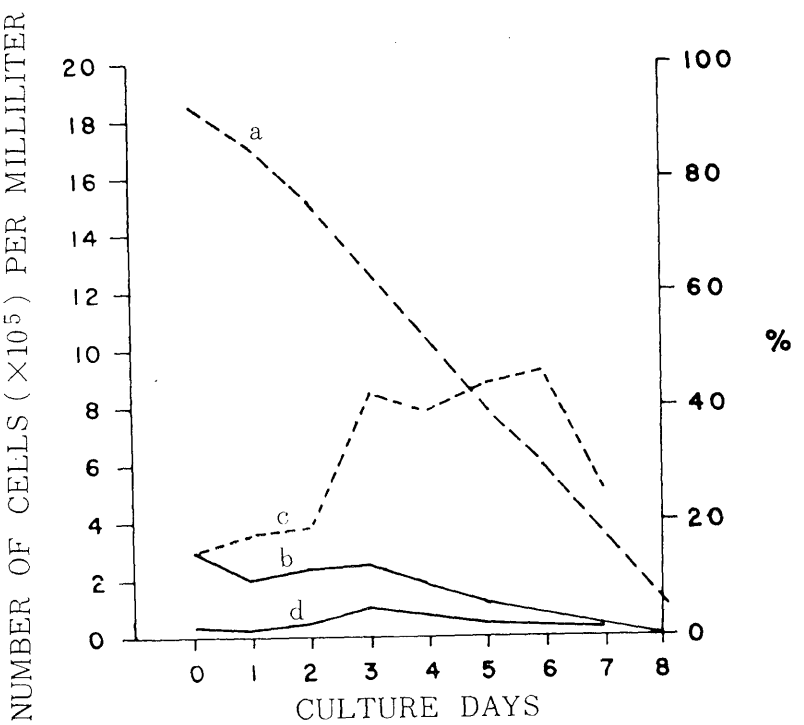

NUMBER OF CELLS (×10⁵) PER MILLILITER
20
18
16
14
12
10
8
6
4
2
0
100
80
60
40
20
0
%
a
b
c
d
0 1 2 3 4 5 6 7 8
CULTURE DAYS

Fig. 6.


NUMBER OF CELLS (×10⁵) PER MILLILITER
20
18
16
14
12
10
8
6
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2
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100
80
60
40
20
0
%
a
b
c
d
0 1 2 3 4 5 6 7 8
CULTURE DAYS

Fig. 7.

rithmic phase of cell proliferation and in medium containing 2% fetal calf serum.

The rate of cell proliferation paralleled the number of immunoglobulin-positive cells in serum containing medium. In serum-free medium, however, immunoglobulin-positive cells remained at a relatively high level during the decline in both population and viability. Caution must be used in setting aside false positive fluorescent reactions of dying cells.

Adult human serum provided results similar to those provided by fetal calf serum.

DISCUSSION

The present study demonstrates that 2% is the minimal concentration of fetal calf serum required in medium RPMI #1640 for spinner cultures of the homan hematopoietic cell lines RPMI #5328, #4265, and #7206. Human serum was a suitable supplement at a level of 1%. The 3 cell lines differed in their ability to adapt themselves to media with low concentrations of serum (unpublished data).

During the logarithmic phase of cell growth, there was a parallel increase in cellular protein and a decrease of the protein in the medium. In media containing 10 and 2% serum respectively, however, the cells did not always consume the expected amount of serum protein during cell proliferation. These observations suggest that the cells were absorbing some components of proteins or were incorporating the products of proteolysis[13]. In the declining phase of the cultures, the protein content of the medium increased. Since there was a tendency for the total protein content of the whole culture to decrease, the changes in the cell proteins may have been due to proteolysis rather than absorption of proteins into cells.

The incorporation of tagged protein components from the medium into cellular protein has indicated significant utilization by certain cell lines[14]. Nevertheless, the contribution of dialyzed protein as an added nitrogen source was very minor for HeLa cell cultures[15], and the replacement of serum proteins with other kinds of macromolecules unrelated to cell nutrition was successful in some instances[16]. The data presented are far from uniform, despite the similarity in the experiments. Author's experimental results suggest the both the entry of proteins into cells in the stage of protein synthesis and the release of proteins from cells in the stage of degeneration.

Growth rate, change in cell viability, and the presence of immunoglobulin were studied comparatively in cultures supplemented with various concentrations of serum. Immunoglobulin production was proportional to the rate of cell proliferation in medium containing serum. In the medium containing serum at a low concentration, the ratio of

immunoglobulin-positive cells to total viable cells rose to an extremely high level, and the total number of cells producing immunoglobulin showed a maximal increase on the 4th day of culture, despite a reduction in growth and viability. If immunoglobulin is released into the medium from degenerating cells, a high percentage of immunoglobulin-positive cells may result from these cells continuing in the stage of immunoglobulin production and storage for a long time prior to death, since their presence in culture medium containing low levels of serum could be due to release from dead cells.

The more immunoglobulin production by cells in the logarithmic phase of cell proliferation suggests that vigorous cell metabolism tends to increase immunoglobulin production. It would seem that non-producing cells that react to an antibody reagent might have some potential capability for producing immunoglobulin. Immunoglobulin-producing activity has been shown to vary during culture[5,8,9]. Furthermore, the immunoglobulin production by the cells in vitro may apparently related to their metabolism and protein synthesis apart from cell division, but it was difficult to evaluate the functional state of cells in any specific stage of cultivation.

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REFERENCES

1. MOORE, G. E., ITO, E., ULRICH, K., SANDBERG, A. A.,: *Cancer*, 19, 713, 1966.
2. MOORE, G. E., GERNER, R. E., FRANKLIN, H. A.,: *J.A.M.A.*, 199, 519, 1967.
3. MOORE, G. E., GRACE, J. T., CITRON, P., GERNER, R. E., BURNS, A.,: *New York State J. Med.*, 66, 2757, 1966.
4. FAHEY, J. L., LAWRENCE, M. E.,: J. *Immunol.*, 91, 597, 1963.
5. HIRSHHORN, K., BACH, F., KOLODNY, R. L., FIRSHEIN, I. L., HASHEM, N.,: *Science*, 142, 1185, 1963.
6. FAHEY, J. L., FINEGOLD, I., RABSON, A.S., MANAKER, R. A.,: *Science*, 152, 1259, 1966.
7. WAKEFIELD, J.D., THORBECKE, G. J., OLD, L.J., Boyse, E. A.,: *Fed, Proc.*, 25, 660, 1966.
8. TANIGAKI, N., YAGI, Y., MOORE, G. E., PRESSMAN, D.,: *J. Immunol.*, 97, 634, 1966.
9. FURTH, R. van, SCHUIT, H.R.E., HIJMANS, W.,: *Immunology*, 11, 29, 1966.
10. MOORE, G. E., GERNER, R. E., MINOWADA, J.,: in 21st Annual Symposium on Fundamental Cancer Research, Houston, Texas, 1967.
11. MOORE, G. E., GERNER, R. E., KITAMURA, H., MINOWADA, J., FJELDE, A.,: in

3rd Annual Leukocyte Culture Conference, IoWA, IoWA 1967.
12. LOWRY, O.H., JOSEBROUGH, N.J., FARR, A.L., RANDALL, R.J.,: *J. Biol, Chem.*, 193, 265, 1951.
13. DUPREE, L. T., SANFORD, K. K., WESTFALL, B. B., COVALESKY, A. B.,: *Exper. Cell Res.*, 28, 381, 1962.
14. TRITSCH, G. L., FLOSS, D. R., MOORE, G. E.,: *Exper. Cell Res*, 42, 523, 1966.
15. EAGLE, H., PIEZ, K. A.,: *J. Biol. Chem.*, 235, 1095, 1960.
16. KATSUTA, H., TAKAOKA, T., HATTORI, K., KAWADA, I., KAWABARA, H., KUWABARA, S.,: *Japan J. Exper. Med.*, 29, 297, 1959.