Partial Substitution of Serum in Hematopoietic Cell Line Media by Synthetic Polymers

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Several synthetic polymers (hydroxyethyl starch, sodium carboxymethyl cellulose, polyvinylpyrrolidone) markedly improved the growth of three human lymphocyte cell lines [Roswell Park Memorial Institute (RPMI) 1348, 1788, and 8098]. Growth was stimulated when each of these polymers was added to RPMI 1640 medium supplemented with only 2% fetal bovine serum. Dextran T-40, T-70, and T-110 varied in their effect on the growth of these cell lines. Dextran T-250 and Haemaccel did not improve cell yields when partially substituted for the serum. The successful partial substitution of polymers for serum was specific for individual cell lines.

Propogation of human hematopoietic cell lines in submerged culture is done in media which contain 5 to 10% fetal bovine serum (FBS; 9). Serum apparently provides nutritional ingredients for growth, a buffering capacity, and a protective action on cells against physical stresses associated with various kinds of agitation required for submerged cultures (12).

The advantages of adding synthetic polymers, especially methyl cellulose, to mammalian and other animal cell-line cultures growing in static or agitated cultures are well known (2, 3, 6, 13, 14; J. C. Bryant, Ph.D. Thesis, Georgetown Univ., Washington, D.C., 1963; D. J. Merchant et al., Bacteriol. Proc., p. 141, 1962).

A variety of different synthetic polymers have been developed for clinical use (1) and thus should be compatible with cultured lymphocyte cell lines.

The advantages of partial replacement of whole serum in the media for hematopoietic cell lines are as follows. (i) To study immunological functions of these cell lines, it is highly desirable to reduce or to eliminate the serum, or at least the globulin fractions from the culture medium. This simplifies separation and study of specific antigens or antibodies and target-lymph cell interaction. (ii) The biological activity of different batches of serum varies greatly in all of the serum batches. (iii) There is an economical advantage, since serum is the most expensive additive in the media. Thus, the aims of this work are to try to reduce or eliminate the serum by adding synthetic polymers.

MATERIALS AND METHODS

Cell lines. Three hematopoietic (lymphocyte) cell lines were used. Roswell Park Memorial Institute (RPMI) cell lines 1348 and 8098 were derived from theuffy coat of individuals with malignant melanoma (10); RPMI 1788 was derived from theuffy coat of a normal individual (8).

Media. The cell lines were maintained and grown in RPMI medium 1640 (9) without antibiotics (basal medium) supplemented with different concentrations of heat-inactivated FBS (Rehatuin F. S., Reheis Chemical Co., Chicago, Ill.). For the maintenance of static seed cultures, 5% (v/v) FBS, was added to the basal medium.

The medium was made in double-distilled water from stock solutions of amino acids, vitamins, salts, and serum. The pH was adjusted to 7.1 to 7.2 by sodium bicarbonate, and the whole medium except the polymers was sterilized by filtration through MF Millipore-GS pads (mean pore size, 0.22 μm) with 293-mm sterilizing filter holders (Millipore Corp., Bedford, Mass.).

Synthetic polymers. The following synthetic polymers were tested: dextrans T-40, T-70, T-110, and T-250 (Pharmacia, Uppsala, Sweden); sodium carboxymethyl cellulose (Edifas B-50; Imperial Chemical Industries, Ltd., Stevenston, Ayrshire, Scotland); hydroxyethyl starch (HES; McGaw Laboratories, Glendale, Calif.); polyvinylpyrrolidones (Periston and Periston-N; Farbenfabriken Bayer A. G., Leverkusen, West Germany); modified gelatin (Haemaccel; Behringwerke A. G. Marburg/Lahn, West Germany). All of these polymers were gifts of the above mentioned companies.

Stock solutions of these polymers were prepared in double-distilled water, sterilized by autoclaving, and added separately to the medium. These solutions cannot be filter-sterilized.

Propagation methods. Maintenance and experimental cultures were grown in 500-ml spinner flasks (model 3002, Belco Glass, Inc., Vineland, N.J.).
The magnetic rotors were kept at about 100 rev/min. Cultures were incubated at 37°C in a special incubator (3). Seed cultures were grown for 5 days and centrifuged at 500 × g for 10 min; cells were resuspended in 200 ml of a test medium. Initial viable cell concentration in cultures was adjusted to 10⁶ to 2 × 10⁶ cells/ml, and the experiments were run for 7 days.

**Cell counts.** Cell counts were carried out in an eosinophil hemocytometer chamber. The viability of cells was assessed by using Trypan Blue (0.1% in Hanks balanced salts solution), a modification of Evans and Schuleman staining method (4). Cell counts are expressed as cell numbers per milliliter of culture.

**Determination of dextrose consumption in the tested culture.** Reducing sugar concentration in culture fluid was determined by the ferricyanide-potassium-ferrocyanide oxidation method (Technicon method for glucose determination, method file N-2b, Technicon, Ardsley, N.Y.).

**Viscosity measurements.** Viscosity measurements of media supplemented with tested polymers, or different concentrations of FBS, or both, were done at 37°C by using a Synchro-Lectric Viscometer (model LVT, Brookfield Engineering Laboratories, Stoughton, Mass.).

**RESULTS**

**Viscosity of the tested media.** The first step in this work was to test the substitution of FBS in the medium by adding different synthetic polymers in such a concentration that the viscosity of the medium would remain approximately similar to the medium supplemented with 5 to 10% FBS. It was found that if the FBS in the medium was reduced to 2%, the desirable concentration of the additive polymers was 0.1 to 0.2% and the viscosity of the medium was unchanged.

**Effect of dextrans.** The effect of four dextrans (T-40, T-70, T-110, and T-250) on the growth of the three cell lines was tested when these polymers were added to basal medium supplemented with 2% FBS.

The results in Fig. 1 to 3 indicate variation in the effect of dextran T-40 and dextran T-70. Although higher cell populations were achieved when these dextrans were added to cultures of RPMI 1788 and 8098 cell lines, no improvement was observed when added to RPMI 1348 cell line culture. Neither dextrans T-110 nor T-250 supported cell growth when added to RPMI.
1348 and 1788 cell-line cultures. An improvement in cell growth was observed when dextran T-110 was added to RPMI 8098 cell-line culture, whereas no improvement was achieved in the presence of dextran T-250 (Fig. 1–3).

Effect of HES, carboxymethyl cellulose, modified gelatin, and polyvinylpyrrolidones. The effect of adding each of these polymers to basal medium with 2% FBS on cell growth is shown in Fig. 4 to 6. All of these compounds (except Haemaccel) showed a marked increase in cell yield for the three cell lines in comparison with basal medium and 2% FBS.

The addition of Haemaccel to basal medium supplemented with 2% FBS did not improve cell growth.

In another experiment, the addition of 0.2% HES or 0.1% Edifas B-50 to basal medium supplemented with 5% FBS was tested. The growth rate was compared to that in basal medium containing 5 and 10% FBS. Growth curves (not given here) show slightly higher cell yield in cultures with the polymers than in basal medium with 5% FBS but low yield when compared with the growth in basal medium with 10% FBS.

We also found that it was impossible to reduce the serum concentration less than 2%, even if any polymer was added, since a very poor yield was achieved.

Thus, serum concentration can be reduced from 5 to 2% in RPMI 1348, 1788, and 8098 cell-line cultures when 0.2% HES, 0.1% Edifas B-50, 0.2% Periston, or 0.2% Periston-N is added. These results were confirmed in bench and pilot-plant-scale propagations in our mammalian cell plant (11).

Dextrose consumption. None of the polymers tested affects the concentration of the reducing sugars in the basal medium. In Table 1, the dextrose consumption is summarized. Generally, when high cell yield was achieved, a higher dextrose utilization was observed (Table 1).
DISCUSSION

It seems that these findings are strain-specific and have to be tested for each hematopoietic, mammalian, and other animal cell lines.

The function of these synthetic polymers in tissue culture has not yet been determined. Some investigators (2, 3, 6; J. C. Bryant, Ph.D. Thesis, Georgetown Univ., Washington, D.C., 1963; D. J. Merchant et al., Bacteriol. Proc., p. 141, 1962) who have added methyl cellulose to tissue-culture media have suggested that the main role of this compound is as a protective agent. Merchant and his co-workers (Bacteriol. Proc., p.

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**Table 1. Effect of polymers on dextrose consumption**

| RPMI cell line no. | Per cent of dextrose consumption |
|-------------------|---------------------------------|
|                  | 5% FBS | 2% FBS | 2% FBS and 0.2% dextran T-40 | 2% FBS and 0.2% dextran T-70 | 2% FBS and 0.2% dextran T-250 | 2% FBS and 0.2% Haemaccel | 2% FBS and 0.2% Edifas B-50 | 2% FBS and 0.2% Periston | 2% FBS and 0.2% Periston-N |
| 1348              | 74     | 60     | 55                             | 60                             | 55                             | 54                             | 69                             | 74                             | 62                             | 75                             | 74                             |
| 1788              | 75     | 51     | 61                             | 61                             | 50                             | 49                             | 69                             | 84                             | 50                             | 69                             | 69                             |
| 8098              | 80     | 76     | 85                             | 80                             | 75                             | 75                             | 85                             | 90                             | 77                             | 85                             | 85                             |

* Basal medium is all tested media. RPMI 1640 supplemented as indicated above.
The page discusses the effect of methyl cellulose on mammalian cells. Kuchler et al. (6) succeeded in eliminating the horse serum in their L fibroblast cell-line cultures by adding methyl cellulose. They suggested that the function of the serum proteins and the methyl cellulose is the same, both are bound to the surface of the cells and thus protect the cell from physical stresses. Thomas and Johnson (14) suggested that besides the protective function of methyl cellulose it has a stimulatory effect on their NCTC clone 929 strain L cells in suspended culture.

We assume that the main function of each of the promising synthetic polymers is protection of the cells and may be similar to the protective effect of the mucopolysaccharides, which mammalian cells produce in vitro, bind to the cell surface, and use as a protective agent (15).

An intensive investigation is needed to determine the exact function of these high-molecular-weight substances in lymphocyte cultures. Are they primarily protective, or do they somehow affect the cell's metabolism, or do both occur? In any case, the synthetic polymers may be classified as secondary factors in this fermentation process (7).

We intend to investigate the effect of growing hematopoietic cell lines in media with synthetic polymers on their production of immunoglobulins.

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