Pluronic Polyols in Human Lymphocyte Cell Line Cultures

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Pluronic polyols markedly improved the growth of two human lymphocyte cell lines when added to the growth medium in concentrations of 0.05 to 0.1%. The results of the current studies suggest that, in addition to the protective effect of polyols against mechanical damage of mammalian cells in submerged cultures, the pluronic compounds may also, by lowering surface tension, facilitate transport of metabolites into cells and thus increase the growth rate.

Several investigators have reported on the addition of various pluronic polyols (PI) to mammalian cell culture media. PI F68 or PI F88 was added to mouse LS cell cultures (4), mouse fibroblast cultures (12, 13), and baby hamster kidney cell line cultures (11). The PI, classified as polyoxypropylenepolyoxyethylene condensates, are nonionic surface-active polymers (2).

Large scale propagation of human lymphocyte cell lines (HLC) in submerged cultures has been described in recent years (15). HLC are known to produce immunoglobulins (16), lymphokines (3), and other compounds. Large quantities of HLC cells are required for clinical trials in cancer therapy (9) and for production of antilymphocyte serum (10). Thus, improvement of media for the propagation of HLC is of importance in order to achieve higher cell yields. In the present study the effect of different PI on HLC growth was investigated.

MATERIALS AND METHODS

Cell lines. Two HLC were investigated: Roswell Park Memorial Institute (RPMI) cell line no. 8432, derived from a patient with acute lymphatic leukemia (this cell line was kindly sent to us by G. E. Moore, Denver General Hospital, Denver, Colo.), and Israel Institute for Biological Research (IIBR) no. 1 cell line. The latter cell line was established from the buffy coat of the peripheral blood of a healthy individual.

Medium. The HLOs were maintained and grown in RPMI 1640 medium (8), supplemented with 5% (vol/vol) heat-inactivated fetal calf serum (both the powdered medium and the serum were purchased from GIBCO, Grand Island, N.Y.). The following antibiotics were added to the medium: 100 U of sodium-G-penicillin per ml and 100 μ g of dihydrostreptomycin per ml.

The basal medium was prepared in deionized water and sterilized by filtration through Millipore GS pads (mean pore size: 0.22 μ m).

Pluronic polyols. The following PIs were investi-

gated: F68, F77, F88, and F108. All PIs were a gift from BASF Wyandotte Corp., Wyandotte, Mich.

The PI solutions, prepared in deionized water, were sterilized in an autoclave for 20 min at 15 lb/in² and added aseptically to the medium before use. All the PIs tested mixed well with the culture medium.

Propagation methods. Maintenance of cultures was carried out in the basal medium in 100-ml spinner flasks (model 3002, Bellco Glass Inc., Vineland, N.J.). Experiments were carried out in shake flasks: 50-ml Erlenmeyer flasks, each containing 20 ml of medium under investigation. Propagation was undertaken on a rotative shaker with a 1-inch (2.54 cm) eccentricity at 200 rpm (model V of New Brunswick Scientific Co., New Brunswick, N.J.). All cultures were incubated at 36 ± 1 C.

Cell counts. Cell counts were carried out as described previously (7).

Determination of PI F68. Quantitative determination of PI F68 concentrations in the culture supernatant was done using the method described by BASF Wyandotte Corp. (1).

Surface tension. The surface tension of the medium was determined at 20 C by the method described by Traube and Blumenthal (14) by means of a stalagmometer (Braid and Tatlock Ltd., Chadwell Heath, England).

Results shown are an average of at least two experiments, each performed in duplicate.

RESULTS AND DISCUSSION

Effect of different PIs on growth of RPMI 8432 cell line. The effect of different concentrations of PI F68 and F108 was investigated. The results are given in Fig. 1 and 2.

The addition of 0.5 or 0.1% of the PI under test increased cell growth as compared with growth in the basal medium. In the presence of 0.2% of the polyols the same growth as in the basal medium, or even a significant decrease of growth, was observed.

Determination of PI F68 indicated that there was no change in its concentration during the 7-day cultivation period.

FIG. 1. Effect of Pl F68 on growth of RPMI 8432 cell line. Symbols: (○) basal medium; (△) basal medium with 0.05% Pl F68; (□) basal medium with 0.1% Pl F68; (●) basal medium with 0.2% Pl F68.

FIG. 2. Effect of Pl F108 on growth of RPMI 8432 cell line. Symbols: (○) basal medium; (△) basal medium with 0.15% Pl F108; (□) basal medium with 0.1% Pl F108; (○) basal medium with 0.2% Pl F108.

Similar results were obtained with Pl F77 and F88.

Effect of different Pls on growth of IIBR 1 cell line. The effect of different Pls was investigated on the IIBR 1 cell line. Results given in Table 1 indicate that only in the presence of 0.05 and 0.1% of Pl F68 and F88 were higher cell yields obtained as compared to growth in the

TABLE 1. Effect of pluronic polyols on growth of IIBR 1 cell line

Pluronic compound added	% (vol/vol)	Highest viable cell count per milliliter of culture ($\times 10^5$)
Basal medium	0	10
Pl F68	0.05	15
	0.10	14
	0.20	9
Pl F77	0.05	10
	0.10	9
	0.20	8
Pl F88	0.05	14
	0.10	14
	0.20	8
Pl F108	0.05	9
	0.10	7
	0.20	7

basal medium. Poor growth was observed in the presence of 0.2% of these Pls and in the presence of 0.05 to 0.2% of Pl F77 and F108.

It appears that the effect of different Pls may be cell line-specific and may have to be determined individually for each lymphocyte, mammalian and other animal cell lines.

The experiments described above with both cell lines were carried out over a period of several months. Numerous passages of the cells were made in media containing one of the above four Pls. No detrimental effects on cell growth or changes in cell morphology (as determined by light microscopy) could be attributed to the presence of Pls in the growth media.

Surface tension. To investigate the role of these polyols in the HLC culture, the surface tension of the basal medium supplemented with different concentrations of the Pl was determined. Results are given in Table 2. The addition of the Pl lowered the surface tension of the medium. A direct correlation between the Pl concentration and the decrease of the surface tension was observed. On the other hand, no correlation between the molecular weight of the Pl, viscosity of the Pl, and the lowering of the surface tension of the medium was found.

A similar effect of surface active agents has been demonstrated in cultures of the fungus *Claviceps paspali* (6). In these fungal cultures glycols and Tweens lowered the surface tension of the medium and thus improved transport of metabolites into the fungal cells.

TABLE 2. Effect of pluronic polyols on surface tension of basal medium

Medium composition	Avg molecular weight ^a	Typical Brookfield viscosity at 77 C (centipoise) ^a	% added (vol/vol)	Surface tension (dynes/cm)
Deionized water			0	72.8
			0	74.7
Pl F68	8,350	1,000	0.05	64.5
			0.10	63.1
			0.20	62.0
Pl F77	6,600	415	0.05	61.7
			0.10	59.1
			0.20	57.9
Pl F88	10,800	2,300	0.05	67.6
			0.10	66.0
			0.20	64.5
Pl F108	14,000	8,000	0.05	66.0
			0.10	63.0
			0.20	62.0

^a Data were taken from reference 2.

In previous studies it was assumed by the investigators that Pl, when added to animal cell culture media, protected cells against damage due to excessive agitation or air sparging in submerged cultures (4, 11). We suggest that in addition to the protective effect of the Pl on the mammalian cells in submerged cultures the Pl in the medium may increase transport of metabolites into the cells, and this may result in higher cell growth rates. These Pls may be classified as secondary factors in the mammalian cell fermentation process (5).

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