PRODUCTION OF FOOT-AND-MOUTH VACCINE: FILTERABILITY OF CATTLE SERUM TREATED WITH POLYETHYLENEGLYCOL AND ITS EFFECT ON CELL GROWTH

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Summary. **The effect of cattle serum containing foot-and-mouth disease antibodies, on the growth of BHK-21 Clone 13 cells grown in suspension was studied before and after treatment with 2, 4, 6 and 8% polyethyleneglycol (PEG). The filtering capability of growth medium supplemented with cattle serum, previously treated with PEG, was also investigated with respect to passage through 0.22 µm membranes. The optimum concentration of PEG was 2% for reasons of economy, high filterability and maintenance of cells.**

The line of baby hamster kidney fibroblastic cells described by MacPherson and Stoker *(9)* adapted to suspension cultures *(5)* is the most utilized in producing antigens for the preparation of foot-and-mouth disease (FMD) vaccines *(11)*. Cell growth *in vitro* requires a complex range of molecules some of which are present in animal sera *(3)*. In the case of FMD antigen production, cattle serum is widely used in the growth of BHK cell cultures *(12)* and it is essential that such cultures maintain susceptibility to the FMD virus. Because the serum taken from live animals may be the vehicle for infectious agents and toxics factors that endanger the growth of cell cultures, every batch of serum should be adequately controlled.

Warrington and Morgan *(16)* demonstrated that the treatment of cattle serum with polyethyleneglycol (PEG) selectively precipitated the antibodies and the high molecular weight proteins. Barteling *(4)* used 8% PEG to treat sera taken from cattle vaccinated several times with FMD virus to remove the anti-FMD antibodies, and observed that the culture media supplemented with the treated sera continued to promote the growth of BHK-21 Clone 13 cells in suspension. Monolayer cell cultures maintained in growth medium containing sera treated with 8% PEG retained susceptibility when challenged with FMD *(1)*.

Commonly, FMD-vaccine-producing laboratories are located in areas where the disease is endemic and face difficulties related to their need to sterilize large volumes of culture medium supplemented with cattle serum, by means of membrane-type filters. Moreover, they have the drawback that the available cattle sera may contain high levels of FMD virus antibodies.

This study seeks to determine what proportion of PEG is most suitable to treat sera from cattle vaccinated with FMD vaccines with respect to filterability through 0.22 µm membranes and cell growth in roller bottles.

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MATERIALS AND METHODS

Bovine serum: It was prepared from blood taken from cattle at the moment of slaughter in the abbattoir (Bagé, Rio Grande do Sul, Brazil) by cutting the carotid artery. The blood was collected in sterilized containers holding a solution of 100 IU of penicillin/ml and 0.15 mg of neomycin/ml. The coagulated blood was later cut into small pieces and left to stand for 24 hours at 4°C in plastic containers. The serum was separated from cell debris and coagulated blood waste by continuous centrifugation.

Treatment of the serum with PEG: Aliquots of 1000 ml of serum were respectively mixed with 2, 4, 6 and 8% PEG (Polyethyleneglycol 8000 - Carbowax) dissolved in 0.15 M sterile saline solution. The mixture was agitated continuously for 60 minutes at 4°C, then immediately left to stand for another 60 minutes at 4°C (*4*). The supernatant was then removed and filtered through a 0.22 µm millipore membrane. Osmolarity and pH were subsequently measured. The level of specific antibodies in the treated sera for the O, A and C types of FMD was determined by the mouse protection test (*6*) and immunoelectrophoresis (*13*).

Cell culture medium with serum: Aliquots of serum before and after PEG treatment were blended with culture medium prior to sterility and cell growth tests. The culture medium used (MEM) to conduct the described study was the same as that used routinely at the Pan American Foot-and-Mouth Disease Center (PANAFTOSA) Vaccine Production Plant.

BHK-21 Clone 13 cells: The study used low passage BHK-21 Clone 13 cells adapted to suspension culture and maintained in liquid nitrogen in 1 ml volumes at a concentration of 5.0 x 106 cell/ml in MEM medium containing 20% inactivated cattle serum and 10% glycerol. The cells were thawed and grown in 2-liter glass vessels containing 1 liter of growth medium. The cells were incubated at 37°C with magnetic stirring and were aereated by means of nonabsorbent cotton filters to maintain the pH.

The capacity to promote the cell growth of the serum was assessed by means of microscopic observation of cell morphology and quantity. Analysis of cell viability was based on the growth curves of five successive passages and readings were taken at 24 and 48 hours intervals post-inoculation of the medium. The initial reading was adjusted to 0.5 x 106 cell/ml.

RESULTS

Filterability of the serum treated with PEG: Table 1 shows the results of the filterability of the serum. It is observed that the filtering of the serum treated with 2% PEG or more yielded five times more than the untreated serum of the same batch.

Tables 2 & 3 show that no changes in the pH or osmolarity values were recorded in the culture medium supplemented with the serum before and after PEG treatment. The tonicity standards of bovine fetal serum vary between 260 and 340 m Osm/kg H_2O.

The cartridge type of 0.22 µm membrane filter system utilized at the PANAFTOSA Vaccine Production Plant permits filtering of about 200 liters of culture medium supplemented with 8% whole untreated cattle serum. However, up to 1500 liters of culture medium could be filtered when it contained the same amount of 2% PEG-treated serum (unpublished data).

FMD antibodies: The antibody levels determined from the mouse protection (Table 4) and immunoelectrophoresis (Fig. 1) tests on serum treated with 2% PEG were similar to those in untreated serum whereas treatment with 8% PEG removed most of the FMD specific antibody.

TABLE 1.Filterability (0.22 µm membrane) of cattle serum before and after PEG treatment.

Treatment with PEG (%)	Volumen filtered (ml)
0	5
2	25
4	25
6	30
8	40

TABLE 2. pH of the serum and of the culture medium with serum before and after PEG treatment.

Treatment with PEG (%)	Serum	Medium with serum
0	7.2	7.52
2	7.3	7.52
4	7.4	7.52
6	7.4	7.52
8	7.7	7.52

and inhibiting the action of proteolytic enzymes, and the globulins promote cell growth by stimulating the synthesis of DNA, RNA and proteins (*8*). Likewise, other polypeptides and hormones produce diverse effects on cells, while insulin regulates the consumption of glucose and amino acids, and may be mitogenic. Hydrocortisone acts on the binding and proliferation of cells (*7*).

Studies of the filterability of serum with varying percentages of PEG (Table 1) showed that the treatment with 2% PEG was the lowest quantity capable of increasing the filterability in comparison with untreated serum. On the other hand, treatments with 4 & 6% PEG did not provide greater yield, although an excellent yield resulted from treatment with 8% PEG. However, the latter treatment may cause problems in the production of FMD virus when it is incorporated into the medium for growth of BHK cell cultures in roller bottles (*14, 15*). Therefore, it follows that FMD vaccine production laboratories could utilize treatment of serum with 2% PEG, thereby lessening the difficulty of sterilizing large volumes of culture medium, supplemented with cattle serum, by passage through 0.22 μm membranes.

TABLE 3.Osmolarity (m Osm/kg H2O) of the serum and of the culture medium with serum before and after PEG treatment.

Treatment with PEG (%)	Serum	Medium with serum
0	304	350
2	321	351
4	339	351
6	362	349
8	392	350

This study also confirmed that the 2% PEG-treated serum, when incorporated into the culture medium, maintained the morphological characteristics and cell growth (Table 4) to the same extent as serum left untreated or treated with 4, 6 and 8% PEG.

Treatment of the serum prior to its blending with the cell medium used in the routine FMD-virus production led to excellent results for the virus produced at the Pilot Plant. It was also shown that 2% PEG treatment did not give problems of cell adherence on the surface of the bottles, nor did it appear to adversely affect viral replications (unpublished data).

The mouse protection (Table 4) and immunoelectrophoresis (Figure 1) tests indicate that treat-

Table 4. Index of antibodies in a serum before and after PEG treatment, by mouse protection test.

Treatment with PEG (%)	O_1 Campos	A_{24} Cruzeiro	C_3 Indaial
0	4.40	1.25	3.60
2	3.60	1.75	2.36
8	0.10	0.10	0.86

Capacity to promote cell growth: Table 5 shows that sera treated with various concentrations of PEG and incorporated into the culture medium maintained cell viability without hampering cell growth throughout five passages.

DISCUSSION

Serum is commonly used as a constituent of growth medium for cell cultures. It is a complex mixture but some of the macromolecules and their activities are recognized. For example, the albumins control cell biosynthesis, protecting against

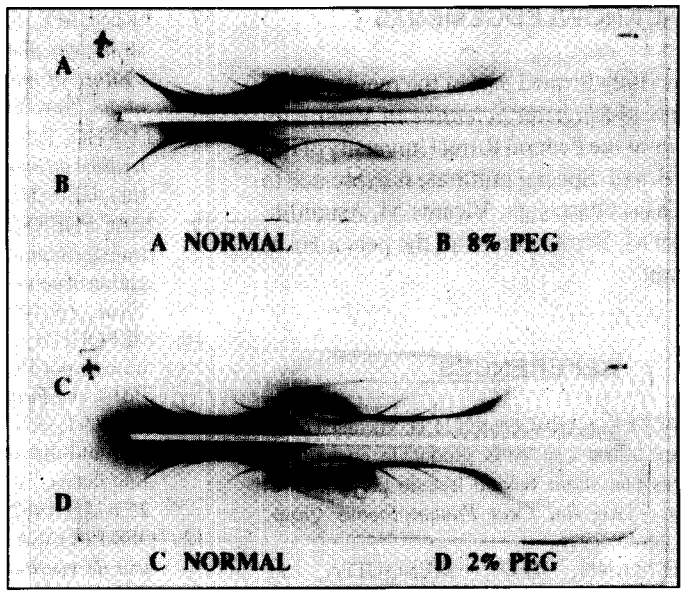

Figure 1.Immunoelectrophoresis in agar gels of cattle sera before and after PEG treatment using as antigen rabbit anti-bovine IgG (central lane). The slides show that there was a total reduction of the levels of detectable antibodies in the 8% PEG-treated serum (B), while in the 2% PEG-treated sera (D) the levels remained similar to those of untreated sera (A & C).

ing the serum with 2% PEG was not capable of removing specific antibodies as did occur with the serum from the same batch when treated with 8% PEG. This therefore precludes its use where cell culture virus is used for diagnostic purposes (*10*). It is believed that the presence of specific antibodies should be as low as possible so that they do not interfere with cell infection by prior neutralization of virus. While this may not be a common problem, there are a number of solutions including the use of serum-free medium, inoculation with sufficient virus to overcome existing antibody (*2*) and using high concentrations of PEG to at least minimize antibody content.

In general, it is concluded that treatment of serum with 2% PEG: (a) appreciably increases the filterability of medium through 0.22 µm membranes; (b) maintain the same capacity to promote cell growth as the untreated serum; (c) although it does not not necessarily remove significant quantities of specific antibodies, neither does it jeopardize the quality of the FMD virus produced in roller bottles for vaccine production, contrary to the observation when 8% PEG is used; (d) enables larger volumes of serum to be handled in a low-temperature asceptic environment, thereby minimizing contamination of the subsequent medium.

Table 5.Growth of BHK-21 cells in suspension in medium supplemented with serum before and after PEG treatment.

	Final Reading (x 10^6 cell/ml)									
Passages	1		2		3		4		5	
% PEG	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
0	1.1	2.3	0.8	1.5	1.9	2.8	0.9	1.7	0.8	2.5
2	0.8	2.1	1.0	1.8	1.4	2.9	1.2	2.8	1.3	2.8
4	1.2	2.7	0.7	2.5	1.4	2.8	1.4	2.4	1.5	2.4
6	1.2	2.5	0.6	1.6	1.7	3.0	1.0	1.4	1.4	2.0
8	0.9	2.1	0.7	1.9	1.2	2.5	0.9	2.3	1.9	2.1

Initial reading adjusted for 0.5 x 106 cell/ml at each passage.

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