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PARTIAL REPLACEMENT OF SERUM WITH PEPTONE AND LACTALBUMIN HYDROLYSATE FOR THE PRODUCTION OF FOOT-AND-MOUTH DISEASE VACCINE IN BHK-21 CELLS

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Summary. — Two batches of experimental media were prepared with Difco-Peptone and Centron-Peptone in combination with lactalbumin hydrolysate (LAH) in Eagle's salts containing 3 amino acids, vitamins and 1 % bovine serum. Both medium batches supported the growth of Razi BHK-21 cells in serial passages and the replication of foot-and-mouth disease (FMD) virus type "A". The infectivity and complement fixing antigen (CFU) titres of the virus were comparable with those in Eagle's medium. The protection indices "C" of the experimental batches of vaccine were highly satisfactory. The experimental media saved up to 90 % of serum in comparison with Eagle's medium.

Key words: *reduced serum medium*; *BHK-21 cells*; *foot and mouth disease vaccine*

Introduction

Active components of bovine serum and their functions had been reported for cell culture work (Barteling, 1977). No availability of good quality serum and its microbial load had been recorded (Panina, 1975). Presently cost of serum has increased due to man power involved in collection, transportation, processing and storing in deep freeze. To solve the problems of serum costs we conducted studies for reduction of serum in FMD vaccine production.

Materials and Methods

Cell lines. Two BHK-21 C-13 cell lines, i.e. BHK-21 C-13 (Glasgow) and Razi BHK-21 C-13 (Suspension) were used at various passage levels.

Control medium. Eagle's MEM-G with 10 % TPB (0.3 % W/V) and 10 % slaughter-house bovine serum was used as growth medium. The maintenance medium was made up of Eagle's MEM-G with 5 % TPB and 5 % serum. For infecting cells in suspension with virus 2 % Polyethylene glycol (PEG 6000)-treated serum was used.

Experimental medium. This medium was formulated with Eagle's salts, vitamins, three amino acids (L-cystine, L-tryptophan, and L-glutamine) and supplemented with lactalbumin hydrolysate (LAH-Difco) and peptone (Difco, USA/Centron Research Lab., India) 3g/L to prepare two experimental batches Nos. 1 and 2. Bovine serum was added to 1 % concentration.

Cell growth in serial passages. Suspension cultures with Razi BHK-21 C-13 cells were put up with 0.5×10^6 cells/ml in closed glass vessels on magnetic stirrers at 37 °C both in control as well as experimental media (Saha *et al.*, 1982). The cells were diluted with fresh medium after 18—20 hr depending upon the live cell count and were allowed to grow further in fresh vessels.

Cell growth curve. Cells grown in serial passages were kept at 4 °C for overnight and diluted with fresh growth medium after discarding the supernatant to have an initial cell count 0.5×10^6/ml at passage Nos. 10, 15, and 20. Live cell count was noted at 24 and 48 hr of incubation. The pH of the culture was adjusted to 7.3 by aeration (Kadoi *et al.*, 1975).

Virus growth curve. Cells grown in serial passages in the experimental media were infected with FMD virus type "A"$_5$ (Saha and Sen, 1987) at different cell passage level with multiplicity of infection 0.1 $TCID_{50}$/cell. Aliquotes of suspension were collected at 6, 12, 18, 20, 24 and 30 hr of post-infection (p.i.). The growth of virus was assayed on the basis of infectivity titre in BHK-21 (Glasgow) cells (Reed and Muench, 1938), complement fixation (CF)-antigen titre (Forman, 1974) and plaque morphology in BHK-21 (Glasgow) cells (Hoskins, 1967).

Preparation of a pilot scale vaccine. Two batches of experimental vaccine were prepared in cells grown in the experimental media Nos. 1 and 2 at passage level 10. Vaccine was also prepared in Eagle's medium. Cells were infected with the virus as mentioned earlier. After 18—29 hr p.i. the virus was harvested. The vaccine was formulated to contain 70 % virus and 30 % alhydrogel in glycocol buffer at final pH 8.7 when inactivated with formalin (0.06 %) for 48 hr at 26 °C. The vaccine was concentrated after 14 days and saponified (Kumar *et al.*, 1979). Potency test of the vaccine was conducted in guinea pigs (Lucam *et al.*, 1964).

Results

In both experimental batches the number of Razi BHK-21 (suspension) cells was always two to three times higher than the initial count upto 20 serial passages. The experimental medium without serum had low cell yield after passage 5 (Table 1). Cell growth followed at different passage levels in the experimental media revealed a maximum cell concentration of 1.9 to 2.1×10^6/ml after 48 hr; it was followed by a phase of decline. Similar cell growth was also achieved in Eagle's MEM-G.

The growth of virus in the experimental media is shown in Table 2. The infectivity and CF-antigen titres of the virus was low at 6 hr p.i. Then it gradually increased and reached to peak after 18-20 hr p.i. Afterwards, the infectivity titre of the virus gradually declined. The CF-antigen titre detected since 6—12 hr p.i. and reached its peak within 18—20 hr p.i. and was maintained more or less up to 30 hr. The growth of virus in each of the experimental medium was comparable with that in Eagle's MEM-G.

Details of vaccine preparation in experimental and control media are shown in Table 2. Infectivity and CF-antigen titres of vaccine virus in the experimental media varied within 6.6 to 6.8 (log_{10}PFU/ml) and 160 (CFU/ml) respectively. Large plaque virus was predominant in suspension cultures. Similar titres and plaque morphology were also observed for the virus in Eagle's medium. The protection indices "C" of the two vaccines prepared in the experimental medium were 4.62 and 3.96 which were on par with those in Eagle's medium. Thus the potency of the vaccines prepared in the experimental media were satisfactory.

Table 1. Growth of Razi BHK-21 C-13 (suspension) cells in two batches of experimental media nos. 1, 2, and in Eagle's MEM-G

Medium	No. of expt.	Initial tial cell count ($\times 10^6$/ml)	Live cell count ($\times 10^6$/ml) in each passage level											
			1	2	3	4	5	6	7	8	9	10	15	20
1	1	0.5	1.2	1.3	1.4	1.6	1.1	1.4	1.3	1.5	1.2	1.6	1.4	1.4
1	2	0.5	1.2	1.4	1.3	1.4	1.3	1.2	1.4	1.4	..3	..5	1.2	1.2
1 (—Serum)	1	0.5	1.2	1.2	1.1	1.0	1.0	0.9	0.8	0.7	0.7	0.7	Discontinued	
2	1	0.5	1.0	1.1	1.2	1.4	1.0	1.2	1.5	1.2	1.5	1.2	1.3	1.2
2	2	0.5	1.1	1.2	1.1	1.3	1.2	1.3	1.3	1.4	1.4	1.3	1.2	1.2
Eagle's MEM-G (Control)	1	0.5	1.2	1.2	1.3	1.4	1.3	1.5	1.2	1.4	1.2	1.3	1.2	1.3

Table 2. Growth of FMD virus type "A" and production of vaccine in Razi BHK-21 C-13 (suspension) cells in experimental media no. 1, 2, and Eagle's MEM-G

No. of expt.	Cell grown in medium no.	Cell passage no.	Titre of virus (log_{10} $TCID_{50}$/ml and CRU/ml) at different hours of post-infection						Titre of vaccine virus		Guinea pig "C" index
			6	12	18	20	34	30	Log_{10}PFU/ml	CFU/ml	
1	1	80/10*	5.1 [<40]	5.7 [>80]	6.8 [160]	6.9 [160]	6. [160]	6.1 [160]	6.8	160	4.62
2	2	80/10*	5.8 [<40]	5.6 [80]	6.5 [160]	6.8 [>160]	6.5 [160]	5.9 [<160]	6.6	160	3.96
3(a)	Eagle's MEM-G	95	5.1 [>40]	5.6 [80]	6.6 [<160]	6.9 [160]	6.3 [160]	6.1 [160]	6.9	160	3.61
(b)	Eagle's MEM-G	120	—	—	—	—	—	—	6.9	160	4.46

Note: Figures in square brackets denote CFU/ml.
* Cell passage number in the experimental media.

Discussion

Complete serum free medium is a complex solution containing a number of chemicals (Griffiths, 1972; Tomei and Issel, 1975; Keay, 1975 and 1977). To make the medium as simple as possible serum was added at the concentration of 1 %. This minimum quantity of bovine serum was considered to provide some of the necessary constituents (Patterson and Maxwell, 1973). Both experimental media supported cell growth in serial passages. The same solutions in the absence of serum failed to support cell growth after 5 passages. Barteling (1977) recommended to add 1—2 % serum to the peptone — LAH medium for better cell yield. In this study the growth of cells in both experimental media was slightly lower as stated by Barteling (1979) who used peptone and LAH (3 g/L each) and automatic pH controlled incubator. However, the experimental media could produce 2×10^6 cells/ml which is essential for FMD vaccine production (Capstick *et al.*, 1967). The precise role of the peptone is unknown. Trace components of serum may be present in the peptone which play an important role in cell growth (Keay, 1976). The peptone and LAH contain all 16 amino acids which are available in the bovine serum (Nagle *et al.*, 1963; Patterson and Maxwell, 1973) fulfilling the partial replacement of the serum.

Growth curve studies of the virus had revealed the exact time for harvesting virus for vaccine production. In the experimental and control media the peak titres of virus were obtained after 18—20 hr p.i. Similar interval was also necessary for harvesting the virus with high titre in laboratory scale (Capstick *et al.*, 1967) and in fermentor scale (Moussa *et al.*, 1974; Freseura, 1975). The vaccine prepared in all media were classified under category No. 1, i.e. very satisfactory (Lucam *et al.*, 1965).

Our experimental media were found suitable for cell and virus growth. They may save 40 L serum per one lakh doses of monovalent vaccine; in addition, they will solve problems with microbial contamination of serum to a great extent.

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