VACCINES AGAINST FOOT-AND-MOUTH DISEASE IN VIRUS PRODUCED IN CELL CULTURES WITH BOVINE SERUM TREATED WITH POLYETHYLENEGLYCOL (PEG)*

Daniel Abaracón**, Homero Giacometti**

PRELIMINARY REPORT

Barteling (4) described the use of serum from cattle vaccinated against foot-and-mouth disease (FMD), from which antibodies and other factors which inhibit the replication of virus had been removed by precipitation with PEG. The author used serum in varying concentrations in culture medium to grow BHK-21 cells in suspension and found that its effect on promoting cell growth was nearly equal to similar serum without PEG treatment. He also reported that it was possible to inoculate virus in cell grown in culture medium containing PEG-treated serum, without replacement of the growth medium. This latter method simplifies the process of antigen production (4).

Our preliminary experiments have focused on three aspects of the use of PEG-treated serum in cell cultures:

a) Elimination of antibodies in sera obtained from slaughterhouses located in areas where FMD is endemic;

b) Cell growth using serum divided into one portion treated with 8.0% PEG and the other portion without treatment; and

c) Production and assays of efficiency of inactivated vaccines prepared with virus replicated in BHK-21 C 13 cells in suspension, with media with PEG-treated serum and without serum.

Table 1 shows the neutralization titers (7) of the sera before and after treatment with PEG. Similar results were obtained with other batches of serum.

No growth differences were found between cell culture suspensions using either PEG-treated or non-treated serum.

The use of PEG-treated sera has already been incorporated into our routine work with cell cultures used for virus titration and for tests to determine the absence of virus infectivity in inactivated vaccines. The susceptibility of the PEG-treated cultures to FMD virus is greater than that of cultures made with the same sera without treatment.

Two trivalent vaccines were prepared, one (Vaccine A) according to the standard technique using cells grown in normal serum, cooling, sedimentation, resuspension of cells in new medium without serum and infection with virus; and the other (Vaccine B) prepared from virus replicated in cell cultures without cooling and without replacement of the growth medium which contained 5.0% bovine serum treated with PEG.

The same seed virus in the same multiplicity of infection was used for both vaccines; the virus produced were submitted to the same treatment with 1.0% chloroform under

* Polyethylene Glycol 6000, J.T. Baker, New Jersey 08865.

** Pan-American Foot-and-Mouth Disease Center, Caixa Postal 589-ZC-00, Rio de Janeiro, RJ, Brazil.

strong agitation, clarification by Seitz K2 filters and inactivation with binary ethylenimine (BEI) (1, 2).

Both vaccines were prepared as follows: the monovalent virus suspensions (Table 2) were inactivated, tested for non-infectivity and mixed. Aluminum hydroxide gel (2.5% Al_2O_3) was then added and the mixture agitated for a few hours. After sedimentation of the aluminum hydroxide, suficient supernatant liquid was eliminated so that each 5 ml dose of trivalent vaccine contained: a) the equivalent of 2 ml of viral suspension of each type; b) 2 ml of $Al(OH)_3$; c) 5 mg of saponin P_3*, d) 0.25 ml of neutral glycerine and e) Lilly merthiolate in a final concentration of 1:30,000.

The efficacy of both vaccines was evaluated by the mouse protection test (MPT) (6) in twenty 15-month old Hereford cattle, which had not been vaccinated and were free of antibodies against FMD before vaccination. These animals were raised and kept on a farm where FMD had not occurred for 12 years. Cattle identified with numbers 1 to 9 were vaccinated with Vaccine A, produced according to the standard method (Table 3); and cattle numbers 10 through 20 with Vaccine B, containing PEG-treated serum (Table 4).

No significant differences were observed between the groups in terms of the percentage of animals considered protected (MPT ≥ 2.0), nor in terms of the expected percentage of protection (8).

Treatment of serum with PEG thus appears to be very promising for the industrial production of vaccines with virus replicated in cell cultures in suspension. The cell cultures can be inoculated with the virus at the optimum point of cell growth without going through the stages of cooling, sedimentation and replacement of the cell growth medium by medium without serum. The elimination of these

* Food Industries, Ltd., Walton-on-Thames, Surrey, England.

TABLE 1 - *Neutralization titers against foot-and-mouth disease virus of serum before and after treatment with PEG*

	Type of virus		
	O_1	A_{24}	C_3
Before treatment[a)]	3.1*	3.1	3.0
After treatment[b)]	0.8	0.5	0.8

* Microneutralization in IBRS-2 cells (7).

a) Serum centrifuged and filtrated by Seitz EKS filters.

b) The same serum treated with 0.8% PEG for 1 hour at 4° C, centrifugated and filtrated through Seitz EKS filters.

TABLE 2 - *Characteristics of antigens produced in BHK-21 cells in suspension by conventional methods and with PEG-treated serum*

Type of virus	Antigen titer	
	$CCID_{50}$/ml*	CF **
Vaccine A[a)]		
O_1	$10^{7.2}$	1/10
A_{24}	$10^{7.2}$	1/11
C_3	$10^{7.3}$	1/12
Vaccine B[b)]		
O_1	$10^{7.5}$	1/13
A_{24}	$10^{7.4}$	1/14
C_3	$10^{7.3}$	1/12

* Cell culture infectant dose 50% per ml.
** Complement fixation titer using 3 units of complement and 90 minutes serum and virus contact.
a) Conventional procedure with normal serum.
b) Assayed procedure with PEG-treated serum.

stages presents several advantages for industrial production, due to increased production capacity, since less time is required for each cycle of virus production; use of less culture medium; and the savings in cells which normally are lost or damaged through the process of cooling and resuspension.

Small quantities of serum always remain in vaccines produced from cell suspension cultures prepared by conventional methods. These small quantities of serum, denatured by the action of formalin during vaccine inactivation, may trigger severe allergic reactions in sensitized cattle (5). Therefore, we believe that inactivants such as BEI must be used which do not act on the serum (3). It is of fundamental importance to determine whether a vaccine containing antigen suspensions with 5.0% bovine serum could act as a sensitizing agent in future vaccinations.

TABLE 3 - *Mouse protection index of cattle 28 days post-vaccination with conventionally produced vaccine*

Cattle No.	Type of virus		
	O_1	A_{24}	C_3
1	3.3	5.3	3.8
2	3.5	4.5	4.3
3	4.5	5.4	≥ 4.0
4	3.0	1.8	2.5
5	2.3	2.8	2.1
6	2.3	4.2	2.0
7	≥ 5.5	≥ 5.8	4.9
8	≥ 5.3	≥ 5.8	5.0
9	4.8	2.7	4.9
Values ≥2.0 over total	9/9	8/9	9/9
Expected percentage of protection	95%	93%	95%

TABLE 4 - *Mouse protection index of cattle 28 days post-vaccination with vaccine produced by the PEG-treated serum method*

Cattle No.	Type of virus		
	O_1	A_{24}	C_3
10	2.3	3.5	1.3
11	≥ 5.3	4.8	4.4
12	4.5	2.8	2.3
13	2.8	5.0	≥ 4.0
14	5.3	4.3	≥ 4.5
15	2.3	4.3	2.0
16	2.1	5.5	1.8
17	≥ 5.5	≥ 5.8	≥ 4.5
18	5.0	≥ 5.3	5.0
19	≥ 5.3	5.5	≥ 4.6
20	≥ 5.3	3.8	≥ 4.6
Values ≥2,0 over total	11/11	11/11	9/11
Expected percentage of protection	95%	95%	90%

REFERENCES

1. BAHNEMANN, H.G.
   Binary ethylenimine as an inactivant for foot-and-mouth disease virus and its application for vaccine production. *Arch. Virol. 47* (1): 47-56, 1975.
2. BAHNEMANN, H.G.; AUGE DE MELLO, P.; ABARACON, D.; GOMES, I.
   Immunogenicity in cattle of foot-and-mouth disease vaccines inactivated with binary ethyleneimine. *Bull. Off. int. Epizoot. 81* (11-12): 1335-1343, 1974.
3. BAHNEMANN, H.G.
   Inactivation of viruses in serum with binary ethyleneimine. *J. clin. Microbiol. 3* (2): 209-210, 1976.
4. BARTELING, S.J.
   Use of polyethyleneglycol-treated serum for production of foot-and-mouth disease virus (FMDV) in growing BHK-suspended cell cultures. *Bull. Off. int. Épizoot. 81* (11-12): 1243-1254, 1974.
5. CAPSTICK, P.B.; PAY, T.W.F.; BEADLE, G.G.; BANDAU, R.; BOGE, A.
   Some studies on allergic reactions to foot-and-mouth disease vaccine in Lower, Saxony, Germany. European Commission for the Control of Foot-and-Mouth Disease. Report of the Meeting of the Research Group of the Standing Technical Committee at the Instituto Zooprofilattico Sperimentale. Brescia, Italy, 24-26 September 1969. FAO. ROME, 1970, pp. 213-218.
6. CUNHA, R.G.; BAPTISTA JUNIOR, J.A.; SERRÃO, U.M.; TORTURELLA, I.
   El uso de los ratones lactantes en la evaluación de los anticuerpos contra el virus de la fiebre aftosa y su significación inmunológica. *Gac. vet. 19* (110): 243-267, 1957.
7. FERREIRA, M.E.V.
   Prueba de microneutralización para estudios de anticuerpos de la fiebre aftosa. *Bltn Centro Panamericano Fiebre Aftosa 21-22:* 17-24, 1976.
8. GOMES, I.; ASTUDILLO, V.
   Foot-and-mouth disease: evaluation of mouse protection test results in relation to cattle immunity. *Bltn Centro Panamericano Fiebre Aftosa 17-18:* 9-16 , 1975.