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Use of Vero Cells for Titration of Rinderpest Virus and Its Neutralizing Antibody (*)

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Primary bovine embryonic kidney cell was successfully replaced by the green monkey cell line (Vero) for the isolation and seroneutralization of rinderpest virus.

The primary bovine embryonic kidney (BEK) cell and its derivative cell lines have long been recognized as the major cell cultures suitable for isolation of rinderpest (RP) virus and evaluation of its neutralizing antibody (NA) and for production of live attenuated RP vaccine (2, 4). Since the introduction of the Vero cell line by Shishido et al. (6) for comparison of biological characters of human measles, RP, and canine distemper viruses, we have used this cell line in a routine serological study of RP. In a comparative study, four batches of live attenuated cell culture of RP vaccine (Kabete O), produced in primary BEK cells by the technique developed by Plowright and Ferris (5), and a virulent strain of virus isolated in a recent outbreak of the disease in Iran were simultaneously titrated in the third subculture of BEK and in Vero cells. The results in Table 1 show somewhat higher titers in Vero cells.

TABLE 1. *Comparative titration of rinderpest virus in Vero and BEK cells*

Material	Titer (negative log ml)	
	Vero	BEK
Rinderpest vaccine		
Batch 691	6.25	5.5
Batch 692	5.75	5.5
Batch 693	5.75	5.5
Batch 694	6.25	6.0
Virulent rinderpest virus RP-1	5.75	5.5

A comparative seroneutralization test was also done by using six unknown cattle sera. Serum samples were heat-inactivated at 56 C for 30' min. The Kabete O virus adapted to both cells and kept at —60 C was diluted in chilled Melnick medium (Earle's saline-lactalbumin hydrolysate containing 2% calf serum free from rinderpest antibody) to contain $10^{3.5}$ $TCID_{50}$/ml. A 1-ml amount of the test dose was added to 1 ml of inactivated serum and diluted in chilled Melnick medium. A cattle serum with a known antibody titer and a serum free from RPNA were included in the test.

Tenfold dilutions of virus were also prepared in chilled Melnick medium. The mixture and the virus dilutions were kept overnight at 4 C. On the following day, 0.2 ml of each virus-serum mixture and 0.1 ml of virus dilutions were added to each of four tubes containing either a confluent sheet of the third subculture

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of BEK or Vero cells grown in Melnick medium containing 10% calf serum.

TABLE 2. *Comparative neutralization test of rinderpest antibody in Vero and BEK cells*

Serum	Serum titer[a]	
	Vero	BEK
1	1/2	1/2
2	1/2	1/2
3	1/6	1/6
4	1/48	1/32
5	1/64	1/64
6	1/256	1/256

[a] The titer is expressed as the dilution of serum neutralizing 100 $TCID_{50}$ of RP virus.

After an adsorption period of 1 hr at 30 C, the maintenance medium similar to the growth medium but containing 2% calf serum free from RP antibody was added and the tubes were kept at 37 C. The results shown in Table 2 were read after 5 days of incubation. At this stage, the test dose of virus showed 100 $TCID_{50}$ per 0.1 ml. The data presented in Table 2 show a close correlation between titers of all sera in both cell cultures. The cytopathogenic effect (CPE) of RP virus in Vero cells is characterized by isolated cell groups rounded and joined together to give the appearance of a bunch of grapes (Fig. 1).

Several strains of RP virus were isolated in Vero cells from the lymph nodes of cattle which died of RP. Two or three blind passages were sufficient for the appearance of the specific CPE of virus in this cell system.

From the results presented here, the advantage of Vero cells for the study of RP virus is evident. The availability of the cell line, i.e., it consistently gives monolayers in 3 days, especially when a large number of serum samples

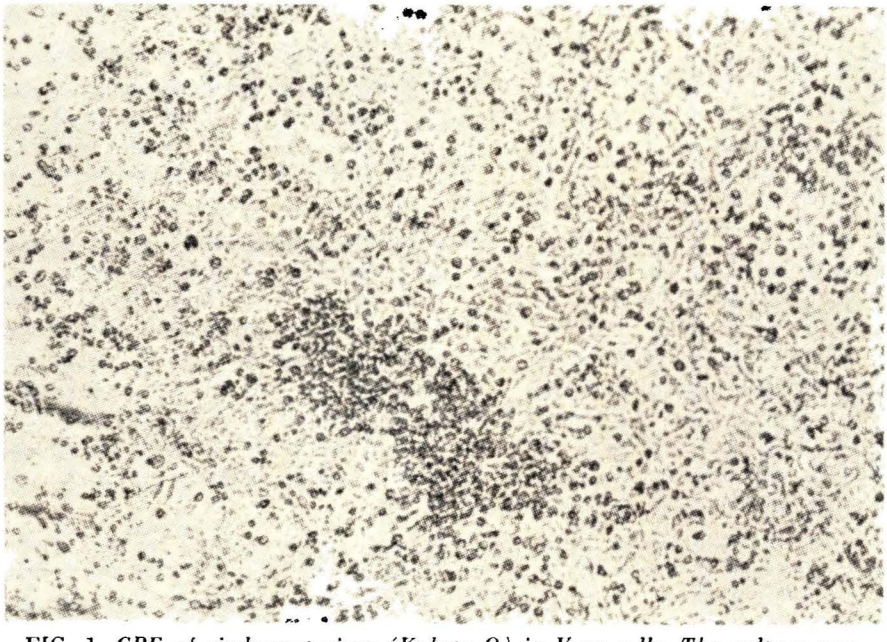

FIG. 1. *CPE of rinderpest virus (Kabete O) in Vero cells. The culture was incubated for 5 days after four passages.*

are to be tested, is a remarkable advantage of Vero cells over the BEK cell line, the growth of which is not consistent from one batch to another. It is also worthy of mention that Vero cells have been shown to be defective in interferon production (1, 3) and seem to be more susceptible to RP virus than the BEK cell line.

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