Plaque Formation with a Phlebotomus (Sandfly) Fever Virus in Baby Hamster Kidney (*BHK* 21) Cells

BABY hamster kidney cell line *BHK* 21, clone 13 (ref. 1), has been used for a plaque assay method for phlebotomus fever viruses. The technique used was a modification of the method described by Russell[2] for herpes virus.

Cells were grown in Eagle's medium containing double amounts of amino-acids and vitamins, 10 per cent tryptose phosphate broth (Difco) and 10 per cent unheated calf serum (ETC). When a confluent sheet of cells was formed, they were dispersed with equal volumes of 0·02 per cent versene solution and 0·25 per cent trypsin (Difco). The cells were suspended in ETC medium; washing was not necessary. They were then counted and the cell suspension adjusted to contain 10^7 cells per ml. The cells can be used immediately or stored at 4° C for up to a week.

Aliquots of 2×10^6 cells in 0·2 ml. ETC were placed in bijou bottles and 0·2 ml. of virus dilution were added. The cell-virus suspension was then shaken gently at 37° C for 30 min. After shaking, 4·5 ml. of ETC were added to each bottle, the contents mixed and poured into 60 mm glass Petri dishes, which were then incubated in a humidified atmosphere containing 5 per cent carbon dioxide at 37° C.

After 5 h of incubation, the ETC medium was removed and the cells were overlaid with 8 ml. of Eagle's medium containing the sodium salt of carboxymethyl cellulose (CMC) in a final concentration of 0·75 per cent and 10 per cent unheated calf serum (ECMC). The overlay medium (ECMC) was made up as follows: 1·3 times normal strength Eagle's medium, 75 ml.; calf serum, 10 ml.; 5 per cent CMC in distilled water, 15 ml. It was of advantage to dissolve 750 mg of CMC in 15 ml. of distilled water in a 150 ml. bottle and autoclave. The Eagle's medium and calf serum were then added. Thorough shaking was necessary to gain a uniform ECMC solution. It was possible afterwards to use a pipette for distributing the overlay medium.

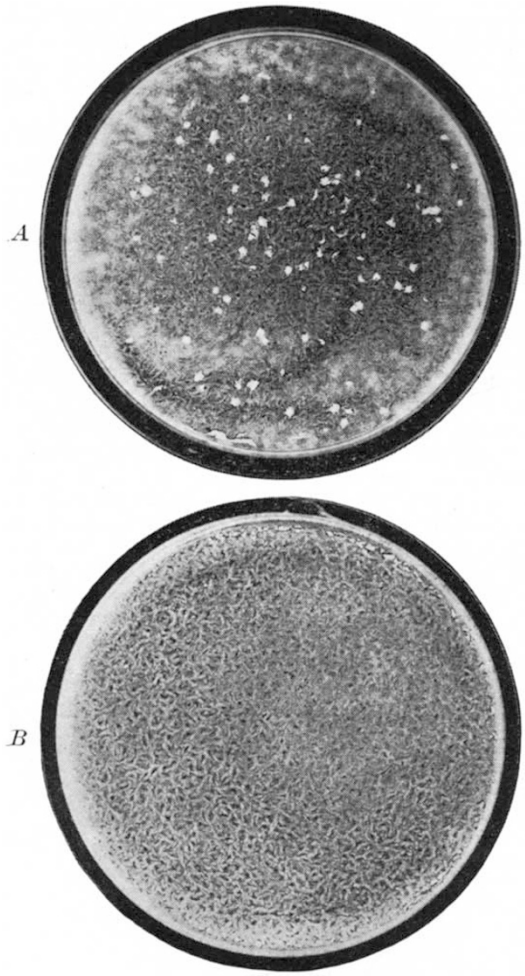

Fig. 1. *A*, Type of plaques produced by Sicilian type of phlebotomus fever virus in *BHK* 21, 10^{-3} dilution of virus, 4 days after inoculation. *B*, Control, cells treated with diluent only

After the addition of the ECMC overlay, the Petri dishes were incubated as before, care being taken not to move them during incubation. 4–5 days later, the ECMC was removed and the cell sheet was either fixed in methanol for 5 min and stained with 1 : 10 Giemsa for 30 min or stained unfixed with 0·1 per cent crystal violet. The stain was then poured off and the plates were gently washed in a bowl of tap water, left to dry and the plaques were counted.

The phlebotomus fever viruses used in this study were:

Sicilian type: 46th mouse passage
Naples type[3,4]: 58th mouse passage

The Sicilian type of virus produced plaques which were irregular in outline and of 1–2 mm in diameter after 4 days of incubation (Fig. 1). The average titre was 7×10^7 P.F.U./ml. as compared with $7{\cdot}4 \times 10^8$ LD_{50} in 2–4-day-old suckling mice inoculated intracerebrally. In a plaque reduction test, 1 ml. of a Sicilian virus dilution containing 300 P.F.U. was mixed with 1 ml. of fourfold dilutions of immune sera prepared in mice against the homologous virus and the Naples virus. The serum–virus mixture was incubated at 37° C for 1 h and then 1 ml. of the mixture was inoculated into *BHK* 21 cells, as already described; two plates were used per serum dilution. The homologous serum gave a 50 per cent plaque reduction titre of 1 : 180, whereas no neutralization was obtained with the Naples serum.

Attempts to produce plaques with the Naples type have so far proved unsuccessful.

A. R. SALIM*

National Institute for Medical Research,
Mill Hill,
London, N.W.7.

* On a scholarship from the University of Khartoum, Sudan.

[1] Macpherson, I., and Stoker, M., *Virology*, **16**, 147 (1962).
[2] Russell, W. C., *Nature*, **195**, 1028 (1962).
[3] Sabin, A. B., Philip, C. B., and Paul, J. R., *J. Amer. Med. Assoc.*, **125**, 603, 693 (1944).
[4] Sabin, A. B., *J. Trop. Med. and Hyg.*, **4**, 198 (1955).

CYTOLOGY

Cytoplasmic Localization of Phytohaemagglutinin in Peripheral White Cells

SALINE extract of the red kidney bean (*Phaseolus vulgaris*) possesses a factor which increases the rate of cell division in cultures of peripheral white cells[1], and a factor which agglutinates red cells[2]. Reciprocal absorption shows that the two factors are separate and the mitogenic moiety has been further characterized as a mixture of proteins, the predominant one having a molecular weight of about 128,000 (ref. 3). The mitogenic extract therefore lends itself to labelling with fluorescent compounds. Such a marker would be valuable in tracing the mode of action of the mitogen(s) since it is still not known whether the leucocyte agglutination, apparently inseparable from the mitogenic activity[4], is also necessary to the mitogenic activity, nor whether the substance acts extra- or intra-cellularly, or both.

To pursue this question a commercial preparation of phytohaemagglutinin (PHA—Burroughs Wellcome Lot No. K4971) was labelled with fluorescein isothiocyanate[5] (FITC—B.B.L. Batch No. 401670) at a fluor-protein ratio of 1 : 40 and cleared of unconjugated FITC by passing it through a 'Sephadex *G*-25' column. The conjugate was