Onderstepoort J. vet. Res., 51, 89–90 (1984)

RESEARCH COMMUNICATION

ACID PRODUCTION BY FLAVIVIRUS-INFECTED VERO AND CER CELL CULTURES

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ABSTRACT

BARNARD, B. J. H., 1984. Acid production by flavivirus-infected VERO and CER cel cultures. *Onderstepoort Journal of Veterinary Research*, 51, 89–90 (1984).

VERO and CER cell cultures infected with flaviviruses produce more acid than non-infected control cultures. Acid production is dependent on the presence of glucose. This phenomenon can be utilized for the titration of flaviviruses.

INTRODUCTION

Cells, metabolizing in a medium containing glucose, produce acid which converts an indicator such as phenol red from red to yellow. Certain cytopathic viruses during their replication cycle effectively inhibit cellular metabolism which is evidenced by reduced acid production in infected cell cultures. This phenomenon was utilized to develop the metabolic inhibition test (Lipton & Steigman, 1955).

During routine cultivation of members of the flavi group of viruses, it was observed that acidity of infected cultures, as indicated by phenol red, was significantly higher than that of non-infected control cultures. Use was made of this phenomenon for developing a colorimetric method for virus titration.

MATERIALS AND METHODS

Viruses and cells

The following flaviviruses were used: Wesselsbron (WSLV), isolated from a calf; Bagaza (BAGV), isolated from a bovine foetus; West Nile (WNV), isolated from a dog, and turkey meningo encephalitis (TMEV), isolated from a turkey. Uganda-S (UG-SV), Spondweni (SPOV), Banzi (BANV) and Ntaya (NTAV) were obtained from Dr B. M. McIntosh of the National Institute for Virology, Sandringham, Johannesburg. Bluetongue virus (BTV) type 3 and Rift Valley fever virus (RVFV), both isolated from sheep, were used as control viruses.

African green monkey kidney cells (VERO) were obtained from Dr Fujita, Tokyo, Japan. The CER cell line was developed by Tsunemasa Motonashi, Nippon Institute for Biological Science, Tokyo, and was obtained from the Veterinary Research Laboratory, Salisbury, Zimbabwe.

Virus titrations

Titrations were done simultaneously in VERO and CER cell cultures in multi-well plastic trays with 96 wells of 15 mm per tray. After inoculation with tenfold dilutions of virus, adsorption was allowed for 30 min at room temperature before the medium was replaced with 0,8 % agarose overlay.

The cultures were incubated in an atmosphere of 3 % CO_2 at 37 °C. A second overlay containing trypan blue, neutral red or phenol red was applied 3–6 days after the first overlay.

Tests were read when plaques were clearly visible on CER cells stained with trypan blue or on VERO cells stained with neutral red. The colours of cultures overlaid with phenol red were recorded daily. All titres were calculated according to the method of Reed & Muench (1938).

RESULTS

Plaques

All the viruses tested formed plaques on both VERO and CER cell monolayers under the conditions described, although the time of appearance, size and clarity varied with the different viruses (Table 1). Plaques were much clearer on CER cells. Both TMEV and BAGV on Day 10 produced plaques which were less than 1 mm in diameter. On VERO cells plaques produced by most viruses were usually poorly visible because of their ill-defined edges and poor staining with both neutral red and trypan blue.

TABLE 1 Virus titres according to plaque titration and acid formation in CER and VERO cell cultures

Virus	CER cultures			VERO cultures		
	Diameter[1] in mm	Titre[2] according to:		Diameter in mm	Titre according to:	
		Plaques	Colour change		Plaques	Colour change
BAN	4,5	5,5	5,5	4,0[3]	6,5	6,5
SPO	3,0–7,0	6,5	6,5	5,0	6,5	6,5
WN	4,0	6,5	6,5	6,0	7,5	7,5
UG-S	2,5	7,5	7,5	4,0	7,5	7,5
WSL	2,5	6,0	6,0	4,0	6,5	6,5
NTA	1,0–2,5	5,5	5,5	4,0	6,5	6,5
BAG	0,5	7,5	8,0	1,0	ND[4]	7,5
ITM	0,5	5,5	5,5	1,0	ND	8,0
RVF	ND[4]	7,5	NY[5]	ND	7,5	NY
BT	ND	6,5	NY	ND	6,0	NY

[1] Diameter of plaques measured on Day 6, except BAGV and ITMV which were measured on Day 10

[2] Expressed as Log_{10} of infective units/mℓ

[3] On VERO cells most plaques were ill-defined, but the titre of BAGV and ITMV could not be determined because of the smallness of the plaques

[4] ND = Not determined

[5] NY = No yellowing

Received 28 October 1983—Editor

Acid production

Acid production, indicated by a yellowing of the overlay, was observed in wells infected with flavivirus only (Table 1). Wells infected with a high multiplicity of RVFV or BTV turned red, while no change in colour was observed in wells with only a few plaques.

Wells infected with flavivirus consistently turned yellow, while those infected with a high multiplicity of virus showed yellowing sooner than wells containing a few plaques only. Yellowing was often observed before plaques were visible and, in wells infected with the 2 viruses which produce small plaques, BAGV and TMEV, yellowing was often observed without visible plaque formation. The difference in colour between infected and non-infected wells was much more marked in VERO-cultures and in cultures exposed to ambient atmosphere for at least 30 min after removal from the incubator. Although the pH of both infected and non-infected wells increased on exposure to the air, the differences remained clearly noticeable for at least 96 h. On prolonged incubation of CER cultures, the pH of non-infected wells decreased to a point where there was no appreciable difference between the colours of infected and non-infected wells. Omission of glucose from the culture medium and overlay resulted in reduced acid production by infected cells, whereas omission of sodium bicarbonate resulted in only a temporary increased alkalinity.

DISCUSSION

The increased acid production of VERO and CER cells infected with flaviviruses seems to be analogous to the behaviour of adenovirus-infected HeLa cells (Fisher & Ginsberg, 1957) and rabies virus-infected chicken embryo cells (Kondo, 1965). In both cases acid production depends on the presence of glucose in the medium. Furthermore, the non-volatile nature of the acid indicates its organic nature.

The increased acidity of infected cultures may be due to an acceleration of cellular metabolism or to an accumulation of acid as a result of inhibition of the later stages of the Krebs citric acid cycle. The increased acidity observed in all the flaviviruses tested indicates that this phenomenon could be an intrinsic characteristic of flaviviruses and a consistent effect in CER and VERO cell cultures. It could thus be utilized for primary identification of newly-isolated virus. The colour change provides an easy means of virus titration with a sensitivity equal to that of plaque titration. The technique, which is performed in the same manner as plaque titration, has some advantages. There is no need for an absolute confluent monolayer and the colorimetric test eliminates the need for time-consuming microscopic observation, with viruses producing minute or indistinct plaques.

The phenomenon as described may possibly be used to perform a colorimetric neutralization test with obvious advantages when flaviviruses, which do not really produce cytophathic changes, or plaques are involved.

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