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Adaptation of Newcastle disease virus (NDV) strains to BHK_{21} (Razi) cell-line*

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Lancaster (1965) emphasized the advantages of using non-avian tissue-culture system to produce Newcastle disease virus (NDV) vaccine. In the present study BHK_{21} (Razi) cell-line was used for assessing the adaptability of 2 lentogenic ('F' and 'LaSota') and a mesogenic (Komarov) strain of Newcastle disease virus since BHK_{21} cell-line is a non-avian cell-line and the cells could be cultivated under suspended animation (Capstick *et al.* 1962).

All the 3 NDV strains were serially passaged in BHK_{21} cells for 30 times adopting routine procedures. At every fifth passage level, the haemagglutination titres (Allan *et al.* 1978) and infectivity titres (Durand and Eisenstark 1959) were assessed as per standard procedures with modification to suit microtechnique. At 30th passage level, the growth kinetics for each virus strain were assessed in BHK_{21} cells by the method described by Kohn and Goldwasser (1957). During passage, the infected cultures were tested for the virus by guineapig erythrocyte adsorption test (Cunningham 1966), fowl erythrocyte adsorption test (Reeve *et al.* 1971), acridine orange staining (Rovozzo and Burke 1973) and fluorescent antibody technique (Purchase 1973).

Initially it required 3 blind passages for NDV 'F' and 2 blind passages for NDV 'LaSota' and 'K' for initiation of cytopathogenic effect (CPE). In the early stages, the CPE observed were rounding and syncytia formation (Fig. 1) resulting in polykaryocytosis followed by extensive grouping and floating of cell aggregates. NDV 'F' and NDV 'LaSota' took more time when com-

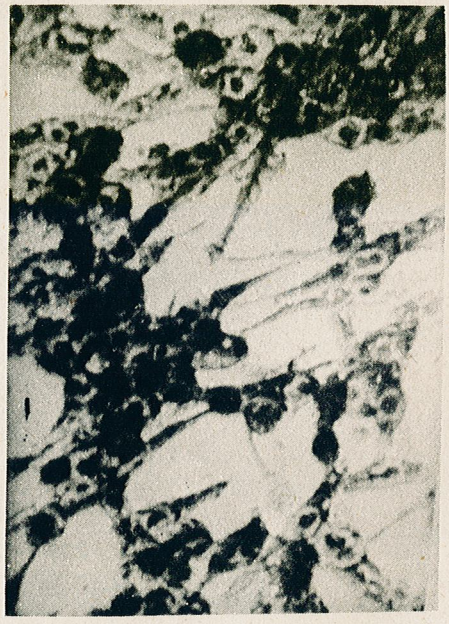

Fig. 1. BHK_{21} (Razi) cell-monolayer - infected with NDV, 24 hr post infection. × 200, Giemsa.

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pared to NDV 'K' for initiation and completion of CPE. Among lentogenic strains 'F' took more time for the induction and completion of CPE, which gradually decreased as the passage level increased.

In virological research especially adaptation studies the presence of virus has to be confirmed. In the present work it was achieved by haemadsorption tests, acridine orange staining and fluorescent antibody technique.

The considerable reduction in haemagglutination (HA) titre of NDV strains during passage in the cell-line and the absence of HA property after certain passage level for 'F' and 'LaSota' (Table 1) clearly showed that NDV lose their haemagglutinins during passage in BHK_{21} cells. Similar findings were recorded by Rossi and Acciarri (1969). However, the infectivity titre gradually increased in relation to the number of passages for all the 3 strains (Table 2). This confirms the findings of French and George (1965). In the present study mesogenic strain 'K' adapted quickly and yielded more titre than lentogenic strains ('F' and 'LaSota') which lead to the conclusion that virulent virus, perhaps would adapt more easily and yield more titre in BHK_{21} cell-line. However, this requires confirmation.

The growth curve studies revealed latent periods of 4-5 hr for NDV 'F' and 3-4 hr for NDV 'K' and 'LaSota'. The maximum virus release in the supernatent occurred at or before 48 hr (Table 3) for all the 3 strains. However further studies are required in respect of number of passages required for stabilization of the virus titre, different vaccines that could be manufactured with stabilized virus and the comparative cost effectiveness of different vaccines besides testing the protection period of each kind of vaccine. It is also necessary to study the effect of these vaccines on production by the vaccinates.

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Table 1. HA titre of NDV strains at various passage levels in BHK_{21} cell-line

NDV strains	HA titre before passage	Reciprocal HA titre at various passage levels					
		Passages					
		5	10	15	20	25	30
'F'	256	16	4	-	-	-	-
'LaSota'	256	16	4	-	-	-	-
'K'	512	16	8	4	4	2	2

Table 2. $TCID_{50}$ of NDV strains at various passage levels in BHK_{21} cell monolayer

NDV strains/	$TCID_{50}/^{50}$ µl at various passage levels (log_{10} titre)					
Passages	5	10	15	20	25	30
'F'	1.50	2.75	3.75	4.50	5.24	6.75
'Lasota'	1.75	3.24	4.24	5.25	5.75	7.24
'K'	2.50	3.75	5.25	6.24	6.75	7.75

Table 3. $TCID^{50}$ of NDV strains released from BHK^{21} cell monolayers at different hours post-infection, at 30th passage level

NDV strains	$TCID_{50}/^{50}$ µ (log_{10} titre)							
	Hours post-infection							
	3	4	5	6	8	24	48	72
'F'	-	-	1.50	2.24	2.75	5.75	6.75	6.75
'Lasota'	-	1.24	1.74	3.24	3.75	6.75	2.24	7.24
'K'	-	1.50	2.24	3.24	4.24	7.24	7.75	7.50

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