Archiv für die gesamte Virusforschung 29, 253—256 (1970)

The Possible Value of Certain Cells for the Propagation of Respiratory Viruses

By

M. VOTAVA[1] and D. A. J. TYRRELL

Common Cold Research Unit, Salisbury, Wiltshire, England

With 1 Figure

Received July 25, 1969

Summary

A strain of HeLa cells, L132 cells and roller tube cultures of human embryo respiratory epithelium were compared with standard laboratory systems for the propagation of certain rhinoviruses, parainfluenza viruses and some others.

1. Introduction

There has been progress recently in the cultivation of "new" respiratory viruses, such as rhinoviruses and human "coronaviruses", and this progress was made by using sensitive cells such as the WI38 strain of human diploid cells and organ cultures of human embryonic respiratory epithelium — the latter seem to be sensitive to many types of respiratory viruses (TYRRELL, BYNOE and HOORN, 1968). It is difficult to obtain enough of such cultures and virus sensitive lines of transformed cells have therefore been sought. Recently it has been shown that certain HeLa cell cultures, maintained with 30 mM $MgCl_2$ in the medium seem to be as sensitive as human diploid cells for the titration and isolation of rhinoviruses (STOTT and TYRRELL, 1968), and that the L132 strain of human embryo lung cells may be better than diploid cells and, at least to some extent, replace organ cultures in the isolation and propagation of coronaviruses (BRADBURNE and TYRRELL, 1969). It has also been suggested that organ cultures of human respiratory epithelium in roller tubes might be as useful as those used previously, which were made in plastic Petri dishes (HARNETT and HOOPER, 1968) and they might require much less tissue and be easier to manipulate.

The object of our studies was to investigate the relative sensitivity of the cultures just mentioned for the growth of some viruses which commonly invade the respiratory tract — respiratory syncytial and parainfluenza viruses, for example — in order to find out whether they might be used for virus isolation and other purposes instead of the cultures now in use.

---

[1] In receipt of a British Council Fellowship. Visiting Worker from the University of Brno, Czechoslovakia.

2. Experimental Results

In the first experiments pools of laboratory-adapted strains of virus were prepared and titrated in our usual preferred cells and also in the cells under study. Titrations were performed using 3 tubes per dilution and 3.2 or 10-fold dilutions.

The rhinoviruses-sensitive HeLa cells (H-HeLa) were those described earlier (STOTT and TYRRELL, 1968) and they and the L132 cells (American Type Culture Collection, BRADBURNE, 1969) were propagated and infected in media described earlier. However, when testing them with influenza and parainfluenza viruses, we washed the cultures and used medium 199 for maintenance. For RS virus we used 2% foetal calf serum. The results obtained with parainfluenza type 2 are given in Table 1. The ratios of the mean titres in the reference culture to that in the test cultures are shown in Fig. 1, which indicates both the cells used and the medium.

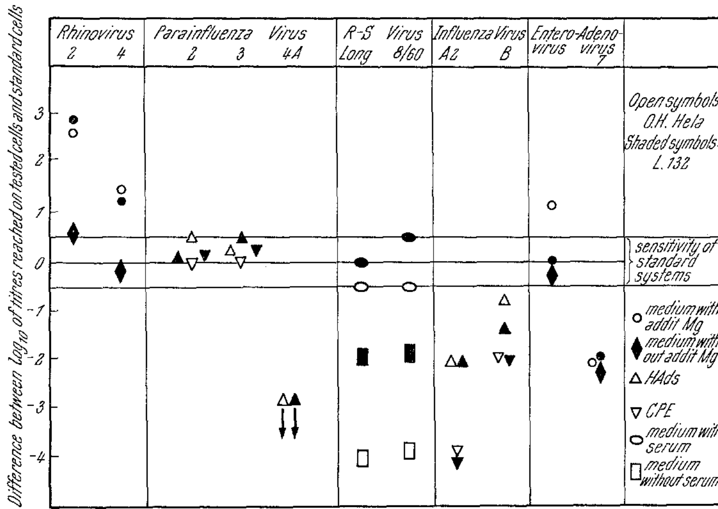

Fig. 1. Ratio of titre found in H-HeLa or in L132 cells to that found in "standard" culture systems. WI38 cells for rhinoviruses, secondary rhesus monkey kidney for parainfluenza and influenza viruses, "Colindale" HeLa cells for RS virus and primary human embryo kidney for adenovirus and enterovirus

It can bee seen that both strains of cells were very sensitive to rhinoviruses — but that the use of a medium with no extra magnesium which was optimal for the production of a cytopathic effect by coronaviruses (BRADBURNE and TYRRELL, 1969) reduced considerably the sensitivity of L132 cells. The cells were very sensitive to parainfluenza viruses 2 and 3, but useless for the cultivation of type 4. The growth of parainfluenza viruses was indicated by the production of large syncytia as well as the appearance of haemadsorption.

The cells seemed to be quite sensitive to infection by respiratory syncytial virus and the syncytia were again clearly visible. On the other hand, although they were fairly sensitive to influenza B virus they were of little value for detecting influenza A virus. The one enterovirus tested was detected as well by the new cells as by the reference system. The titres of adenovirus type 7 were, however, much lower than those in human embryo kidney cells. This may have been because we used calf serum in the medium and terminated the experiment at 7 days when titres in the

kidney cells were already high — more tubes might have become positive if we had kept the continuous cells longer.

Virus adapted to cultures may not behave like viruses in clinical specimens and we therefore made further tests using nasal washings and throat swab fluids in these culture systems. As shown in Table 2, nasal washings collected from volunteers infected with "wild" strains of rhinoviruses and a strain of parainfluenza type 2 were titrated in standard cells, HeLa cells and L 132 cells. In addition, organ cultures of embryonic nasal or tracheal epithelium were made in test tubes. A

Table 1. *Representative Results of Titration of Laboratory Strains of Virus*

Virus used	Log_{10} titre of pool in		
	Monkey kidney cells	H-HeLa	L 132
Parainfluenza 2	5.0	4.8	5.2
	5.1	6.3	5.4
	Mean 5.05	5.55	5.3

All positive tubes were detected by haemadsorption after incubation for 5 days — similar results were detected by examining tubes of HeLa and L 132 cells for cytopathic effect.

Table 2. *Results of Titrating Clinical Specimens Containing Viruses in HeLa, L 132 Cells, or "Organ Cultures" of Pieces of Embryonic Tracheal or Nasal Epithelium in Roller Tubes*

Virus used	Log_{10} titre of specimen in				
	Standard cultures (see Fig. 1)	Cell cultures of		Organ cultures subinoculated on	
		H-HeLa	L 132	4th day	8th day
Rhinovirus 2	0.5	2.5	0.5	3.5	2.25
Rhinovirus 4	0.5	1.2	0.5	neg.	neg.
Rhinovirus 9	1.8	0.5	neg.	3.2	3.2
Rhinovirus 43	0.2	0.2	0.5	2.5	2.5
Parainfluenza 2	0.5	neg.	0.2	2.5	2.5
Influenza B	2.8	N.D.	neg.	2.5	2.5

square about 2 mm across was dropped into 1 or 1.5 ml of Eagle's medium in a stoppered test tube which was incubated at 33° C in a roller drum. The action of cilia could often be observed microscopically, but its presence or absence was of little help in showing the susceptibility of an individual culture or the presence or absence of a virus. However, it was easy to subinoculate the medium from such cultures at 4 and 8 days and to detect specific virus effects in tissue cultures. By this two-stage technique the organ culture infectious dose of these nasal secretions was measured, using 10-fold dilutions and 2 cultures per dilution because there were too few cultures to do more. The results, shown in Table 2, confirm the general conclusions of the preceding experiment and show

that rhinoviruses can be efficiently isolated from clinical specimens in these cultures. It is thought that the rather large differences of titre in different cells are due to uncontrolled fluctuations in the sensitivity of the cells used, particularly the WI38 cells, which probably were below their normal sensitivity in the experiments reported in Fig. 1 and Table 1. L132 cells probably could be used for clinical isolations of parainfluenza 2 but would be of no value for detecting influenza B. The simplified organ cultures were usually far more sensitive than any tissue culture; the only exceptions were that low titres of one strain of rhinovirus were not detected, and that organ culture was no better than monkey kidney cultures for detecting influenza B.

The organ culture fluids were usually positive when collected 4 days after inoculation, as noted before (TYRRELL and BLAMIRE, 1967). Throat swabs from which adeno- and RS virus had been isolated were also tested but were negative in all cultures, presumably because the virus had become inactivated.

3. Discussion

We failed to find a continuous cell which would act as a "universal culture medium" for respiratory viruses. However, we think that the H-HeLa cells and possibly L132 could be as useful as WI38 in the cultivation of rhinoviruses from clinical specimens — it would not be possible to use the same tube of L132 to detect coronaviruses, as this would require a different medium. Either type of cell in a serum-free medium could be used for the recovery of parainfluenza virus and they are apparently sensitive to RS virus. If allround sensitivity is wanted it seems likely that the tube organ cultures of HARNETT and HOOPER (1968) would be most effective — although their efficiency remains to be proved for RS and adenoviruses. HARNETT and HOOPER may have had a rather low rate of virus isolation because the relatively few specimens which they received contained a virus. Fuller investigation of these cultures in a diagnostic laboratory is now required.

Acknowledgements

We would like to thank Mrs. B. Head for technical assistance.

References

1. BRADBURNE, A. F.: Nature (Lond.) **221,** 85—86 (1969).
2. BRADBURNE, A. F., and D. A. J. TYRRELL: Arch. ges. Virusforsch. **28,** 133 (1969).
3. HARNETT, G. B., and W. L. HOOPER: Lancet **1,** 339—340 (1968).
4. STOTT, E. J., and D. A. J. TYRRELL: Arch. ges. Virusforsch. **23,** 236—244 (1968).
5. TYRRELL, D. A. J., and C. J. BLAMIRE: Brit. J. exp. Path. **48,** 217—226 (1967).
6. TYRRELL, D. A. J., M. L. BYNOE, and B. HOORN: Brit. med. J. **1,** 606—610 (1969).

Authors' address: Dr. D. A. J. TYRRELL, Clinical Research Centre, Harvard Hospital Coombe Road, Salisbury, Wiltshire, England.