STUDIES ON THE PATHOGENICITY FOR TISSUE CULTURES OF SOME VIRUSES ISOLATED FROM COMMON COLDS

D. TAYLOR-ROBINSON, R. HUCKER* AND D. A. J. TYRRELL

From the W.H.O. International Reference Centre for Respiratory Virus Diseases and M.R.C. Common Cold Research Unit, Salisbury, Wilts.

Received for publication September 27, 1961

BY using tissue cultures of human embryo kidney (HEK) cells maintained at a lower temperature and pH than is usual, it was possible to recover viruses from the nasal secretions of many patients suffering from common colds (Tyrrell and Bynoe, 1961). A proportion of the viruses were also cytopathic for rhesus monkey kidney (MK) cells and have been called M strains while those strains growing in HEK cells and not in MK cells have been termed H strains. These viruses have been called " Salisbury " strains but recently the name rhinoviruses has been suggested (Andrewes, 1961). Hobson and Schild (1960) showed that two strains of M virus grew in HEp–2 but not in Hela or MS cells. This report describes experiments on several M viruses which demonstrate that they are cytopathic for and multiply in a variety of continuously cultivated cell lines and primary trypsinized cell cultures. The propagation of H strains in a diploid cell line is also described.

MATERIALS AND METHODS

These were in general similar to those described earlier (Tyrrell and Parsons, 1960).

Viruses

Tissue culture fluids were used. Most of the virus strains had been isolated in primary cultures of human embryo kidney cells, and the M strains had, in addition, been passed a few times in secondary cultures of rhesus monkey kidney cells. The passage history of the inocula will be considered again later.

Tissue cultures

Trypsin dispersed primary human cells.—From embryo kidney, embryo liver, amnion.

Continuous cell lines.—HeLa cells obtained from the Public Health Laboratory, Salisbury ; HeLa S3 cloned line ; ERK 1 (Porton) ; MK2 (Porton) and HEp–2 ; a diploid line of human embryo lung fibroblast cells (WI 26 VI) obtained from Dr. Hayflick.

Except when otherwise stated, the cultures were maintained in a roller drum at 33° with 1·5 ml. of medium. The medium contained 0·03 per cent sodium bicarbonate, 0·25 per cent lactalbumin hydrolysate and 2 per cent calf serum in Hanks' saline with antibiotics. The continuous MK2 cell line was maintained with 5 per cent calf serum in initial experiments and the human embryo lung fibroblasts in 10 per cent calf serum and 90 per cent Eagle's medium.

Inoculation and passage

Undiluted tissue culture fluid (0·2 ml.) was inoculated. Sometimes cultures were washed 2–3 hr. later. When a cytopathic effect appeared the culture fluid was harvested and stored at −70°.

* Present address : Glaxo Laboratories, Greenford, Middlesex.

RESULTS

Experiments with M strains

Attempts were made to pass the viruses serially in continuous cell lines. For this purpose cultures were inoculated with virus-containing fluid from human embryo kidney or monkey kidney cultures. If no cytopathic effect was observed in the first experiment, it was repeated with fluids which contained a higher titre of virus, and this often produced a cytopathic effect. Once a cytopathic effect was produced it was generally possible to pass the effect serially in other cultures, and the effect became more marked with each passage. The cytopathic effect was inhibited when cultures were maintained at 36° or at high pH or when the cultures were not rolled. In several experiments the infectivity of the culture fluids was titrated (Parsons and Tyrrell, 1961) and it can be seen from Table I that virus multiplication did occur. Furthermore, the virus recovered

TABLE I.—*The Multiplication of Various Virus strains in Continuous Cell Lines*

Virus*	Tissue culture	Total P.F.U. per culture	
		Added at first passage	Recovered at third passage
H.G.P.	HEp–2	60	1,500
B633	,,	360	15,000
P.P.	,,	approx. 360	1,800
B633	MK 2	360	750
No.	Hayflick	approx. 50	300

* H.G.P., B633 and P.P. are " M " strains.
No. is " H " strain Salisbury /1/51H.

from the last passage was neutralized by specific immune serum prepared against the original virus propagated in HEK or MK cells as shown in Table II. The viruses are arranged in Table II by serotypes (Taylor-Robinson and Tyrrell,

TABLE II.—*Summary of Passage Experiments in Continuous Cell Lines*

Strain	Strain designation‡	Presence (+) or absence (−) of cytopathic effect and number of serial passages in cell line				
		HeLa (PHLS)	HeLa S3	HEp–2	MK 2	Hayflick
H.G.P.	Salisbury/1/57M	+3	−2	+3*	−3	+2*
Harrison	Salisbury/1/55M	ND	ND	+3*	+1*	ND
B632	Salisbury/1/60M	+3	−2	+3*	+3*	+2*
B633.	Salisbury/2/60M	+2	−1	+3*	+3*	ND
Bedwell	Indianapolis/1/60M	+2	−1	+3	−2	ND
JH	ECHO 28	+2	ND	+3*	−2	+2*
B702	Salisbury/3/60M	+1†	−2†	−2	−2	ND
P.P.	Salisbury/4/60M	+2	−2	+3*	+3*	ND
F.E.B.	Salisbury/1/58H	±see text	−1	−2	−2	+3*

‡ Denotes the place, sequence, type of isolation and year of cold.
* The virus from the final pass was shown to be neutralized by a specific immune serum against the virus used to initiate the passages.
† The B702 virus used as inoculum for these cell lines had 3 passages in HEK and 2 passages in MK cells.
ND = Not done.

1962), H.G.P. and Harrison being identical by neutralization tests, and so on. In the third group are included the viruses found to be serologically identical with JH (ECHO 28); the fourth section contains the results with F.E.B., the only H strain tested repeatedly in a variety of cells.

Strains H.G.P., B632, B702 and P.P. produced cytopathic effects in human embryo liver and full-term amnion. The H.G.P. strain produced degeneration in 3 of 4 batches of human embryo lung and in a batch of human embryo skin cultures; degeneration was seen in cells of an epithelial type and not in fibroblast-like cells. All the M strains tested were adapted to one or more of the transformed epithelial-type continuously cultivated cells as shown in Table II. All the strains used as inocula had one or more passages in HEK cells, apart from the Bedwell strain and prototype JH strain which had been isolated in monkey kidney cells; all strains had also 4 or more passages in MK cells except strain B702. Strain P.P., passed 3 times in HEK and once in MK cells, produced no cytopathic effect in transformed cell lines, but after 4 further passages in MK cells the results shown in Table II were obtained. As judged by the number of times cytopathic effect was produced it seems that one strain of HeLa was highly susceptible and another completely resistant, while HEp–2 was quite susceptible and MK2 was rather resistant. ERK cells were used but were found to be rather resistant and were not extensively studied.

It is concluded that the M-type viruses grow and produce degeneration in cultures derived from several human embryo tissues and in a wide range of continuously cultivated cell lines.

There was sometimes difficulty in starting passage in continuous cell lines with virus grown in monkey kidney and human embryo kidney cells. Furthermore, no virus was isolated from nasal washings by inoculation into HEp–2 cells although the same specimens yielded virus in human embryo kidney cells. These observations suggested that continuous cells lines were less susceptible than primary cultures of HEK cells or secondary cultures of MK cells.

Experiments with H strains

The strain F.E.B., which is representative of the H strains, which do not cause degeneration in monkey kidney cells, did not, in general, produce a cytopathic effect in the cells lines used. However, after one blind passage in HeLa cells it produced a transmissible cytopathic effect in these cells. After a total of 3 passages virus was passed back to HEK cells and identified in a neutralization test. However, the particular strain of HeLa cells used was lost and the experiment could not be repeated with other lines of HeLa cells. Nevertheless this experiment seemed to show that, in principle, the H-type strains could also be adapted to continuous lines of transformed epithelial cells, but with greater difficulty than was found with M strains and perhaps only to certain more susceptible lines of cells. No cytopathic effect was produced by H-type viruses (after blind passes) in cultures of monkey embryo kidney. However, the Hayflick line of diploid human embryo lung fibroblasts not only supported the growth of the M strains but also the H strains, F.E.B., No. and Th. (Tyrrell and Bynoe, 1961), known to be serologically distinct (Taylor-Robinson and Tyrrell, 1962). These strains produced a transmissible cytopathic effect, which was focal and developed in 2 to 4 days in the first passage of H strains. After 3 passages the the viruses were identified by neutralization.

Virus production in bottle cultures of cells

Babies' feeding-bottle cultures of HEp–2 cells were infected with an M strain of virus, B633, that had been passed in these cells 3 times previously ; high titres of virus were obtained. The growth curve of virus in such a culture is shown in the figure. The amount of virus obtained at a particular time was about 10-fold greater than from a tube culture and this experiment with HEp–2 cells in a bottle culture indicated that this system could be a useful source of high-titre virus.

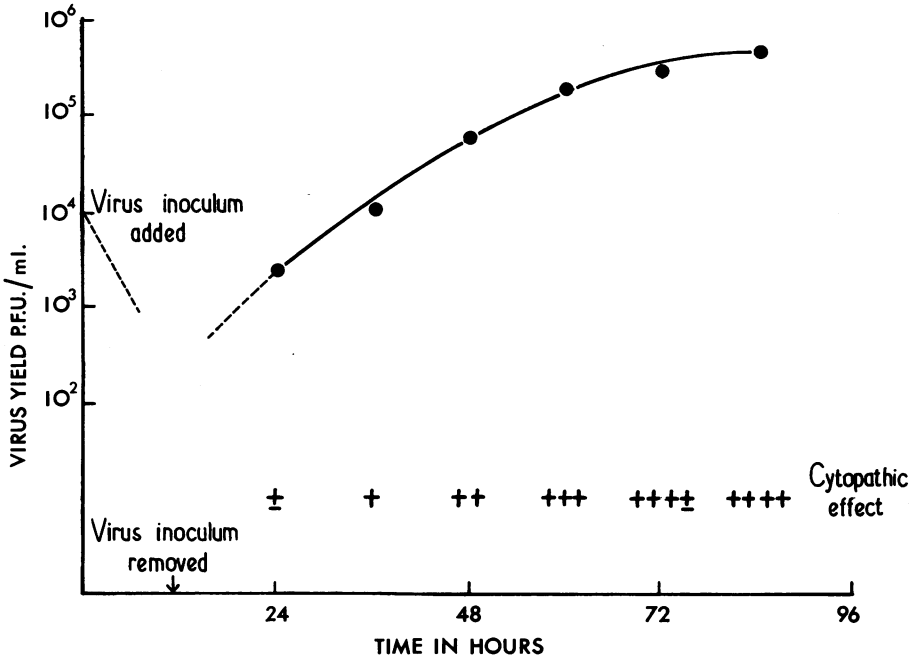

FIG.—The growth curve of B633 virus in a feeding bottle culture of HEp–2 cells. The bottle, with cells on the 6 sides, was rolled at 33°. The virus inoculum was removed after 12 hr. and the culture washed twice with Hanks' solution before 2·0 ml. maintenance medium was replaced. The medium was removed and titrated and replaced by the same volume of fresh medium at 12 hr. intervals.

DISCUSSION

Human embryo kidney cells are difficult to obtain and monkey kidney cells often contain " wild " viruses. The demonstration that rhinoviruses can be adapted to continuous cell lines will facilitate the preparation of large pools of virus which should be useful for serological work such as the establishment of new serotypes and antibody assays. It seems that inoculation of human embryo kidney cells is still the best method of virus isolation.

We were impressed by the wide variations in sensitivity of different cell lines and of sub-lines of HeLa cells. The HeLa cells used were different from those used by Hobson and Schild (1960). HeLa cells resistant to certain strains of virus have been described before (Nakano, 1959 ; Murphy and Armstrong, 1959). This emphasizes the practical value of preserving stocks of virus-sensitive cells

at −70°. The cytopathic effect appeared to be similar to that produced in kidney cells but will be studied further and described in detail later. The diploid cell line is obviously highly susceptible, and this is interesting in view of the relative insensitivity of cultures of primary trypsin-dispersed human lung. Hayflick (personal communication) has also found a cytopathic effect in his cultures after infection with H- or M-type viruses and these virus types have also been adapted to KB cells (Johnson and Chanock, personal communication).

SUMMARY

Representative strains of M-type viruses isolated from common colds have been found to produce cytopathic effects and to multiply in HeLa, HEp–2 and MK–2 cells. They also produce cytopathic effects in human embryo liver and human amnion cells. The F.E.B. strain of H-type virus was found to grow with difficulty in one sub-line only of HeLa cells, but grew readily in a diploid line of human embryo lung cells. Two other H-type viruses also grew in these cells.

We thank Dr. P. J. Wormald (P.H.L.S. Laboratory, General Infirmary, Salisbury) for supplying HeLa cells, Dr. J. C. N. Westwood (M.R.E., Porton, Wiltshire) for supplying HeLa S3, MK2 and ERK1 cells, Dr. C. Placido de Sousa (Wellcome Research Laboratories, Beckenham, Kent) for supplying HEp–2 cells and Dr. L. Hayflick (Wistar Institute, Philadelphia, U.S.A.) for the diploid cell line. The Bedwell strain of virus was kindly sent to us by Dr. R. N. Hull (Eli Lilly, Indianapolis, U.S.A.) and the JH strain by Dr. W. H. Price (Johns Hopkins Hospital, Baltimore, U.S.A.).

REFERENCES

ANDREWES, C. H.—(1961) *Yale J. Biol. Med.* In press.
HOBSON, D. AND SCHILD, G. C.—(1960) *Brit. med. J.*, ii, 1414.
MURPHY, W. H. Jr. AND ARMSTRONG, R.—(1959) *J. exp. Med.*, **110**, 629.
NAKANO, M.—(1959) *Jap. J. med. Sci. Biol.*, **12**, 79.
PARSONS, R. AND TYRRELL, D. A. J.—(1961) *Nature, Lond.*, **189**, 640.
TAYLOR-ROBINSON, D. AND TYRRELL, D. A. J.—(1962) *Lancet*, in the press.
TYRRELL, D. A. J. AND BYNOE, M. L.—(1961) *Brit. med. J.* i, 393.
Idem AND PARSONS, R.—(1960) *Lancet*, i, 239.