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A Comparison of Two Cell Culture Systems for the Primary Isolation of Enteric Viruses

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Several studies have been reported on the comparative susceptibility of various cell cultures for the primary isolation of enteric viruses. The present report gives a comparison of human embryo kidney cells and rhesus monkey kidney cells for the primary isolation of viruses from faecal specimens. Altogether, 148 enteroviruses, covering 21 serotypes, and 20 adenoviruses were isolated. The marked sensitivity of human embryo kidney cells to adenoviruses was again demonstrated. These cells, however, were found to be significantly less sensitive than rhesus monkey cells for the isolation of the enteroviruses encountered. This finding is in conflict with the results of a previous study and is partly explained by the difference in enterovirus composition of the two series. The value of human embryo kidney cells for the primary isolation of enteric viruses would seem to be closely related to the prevalence of adenoviruses and certain coxsackievirus A serotypes in the population studied.

A basic function of a diagnostic virology laboratory is the examination of clinical material for evidence of a virus infection. The isolation of the virus is still of major importance, particularly so for enterovirus infections where diagnosis by serological means (Schmidt et al., 1965, 1967) and the newer diagnostic facilities of immunofluorescence and electron microscopy (Doane et al., 1969) are of limited value.

It follows, therefore, that the provision of the best available cell culture system for isolation of enteric viruses is a matter of considerable importance. For reasons of availability, time and space, most laboratories are restricted in the number of cell systems they can handle. It would clearly be a considerable advantage to have a single, readily available cell culture system that was highly susceptible to all the enteric viruses. Such a system has not yet been shown to be available but a claim that human embryo kidney (HEK) cells come closest to this ideal has been made (Lee et al., 1965).

The purpose of this report is to provide further material in the assessment of the value of HEK cell cultures in the primary isolation of enteric viruses. Comparison has been made with primary isolation in rhesus monkey kidney (MK) cells, the most commonly used cell type for enterovirus isolations and the standard against which other systems are often assessed.

MATERIALS AND METHODS

Specimens

Faecal specimens sent to the virology laboratory for culture were included in this comparison if they were inoculated into both MK cells and HEK cells on the same day (usually the day of receipt).

A 10% suspension of faeces was prepared in Hanks' basal salt solution (HBSS) containing antibiotics. After centrifuging, 0.1 ml of the resulting supernatant constituted the inoculum.

Cell cultures

MK cells were received weekly through the post from the Division of Immunological Products Control of the National Institute for Medical Research, as monolayers in plastic bottles from which growth medium had been removed. On receipt, fresh growth medium was added and the bottles were rolled at 36°C for 2 days. After trypsina-

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tion, tubes were seeded with 1 ml (10^5 cells/ml) of the dispersed cells and incubated while stationary until confluent (usually 4 days), when the growth medium was replaced by maintenance medium.

Fetal human kidneys were trypsinized and tubes seeded with 1 ml of growth medium containing 2×10^5 cells/ml. These were incubated while stationary until confluent (usually 7 days), when the growth medium was replaced with maintenance medium.

When available, cells of different types have been stored frozen in the vapour phase of liquid nitrogen for use when fresh cells are in short supply. During the period covered by this report stored MK and HEK cells were used on a few occasions. Unpublished observations in this laboratory indicate that cell susceptibility to enteric viruses is not likely to be altered significantly by storage under these conditions.

Culture media

The medium used for growth of MK cells was HBSS with lactalbumin hydrolysate (0.5%), ox serum (5%), sodium bicarbonate (0.03%) and antibiotics. The growth medium used with the HEK cells was similar to the above but contained 10% ox serum. The maintenance medium for MK and HEK cells was medium 199 with sodium bicarbonate (0.18%) and antibiotics.

Isolation and identification

For each specimen 2 tubes of each cell type were inoculated. MK cells were incubated (rolled) at 36°C for 14 days during which time a further passage was made in the homologous cell system. HEK cells were incubated (rolled) at 33°C for 21 days during which time at least one further passage was made in the homologous cell system. (Incubation at 33°C has been reported to be optimal for isolation of the more fastidious rhinoviruses (Tyrell & Parsons, 1960); in this study, however, the incubation of HEK cultures at 33°C was adopted as a matter of laboratory convenience.) Cultures were examined periodically for cytopathic effect (CPE). In cultures showing enterovirus-type CPE, agents were identified by the method recommended by Bradstreet (1962) with the enterovirus serum pools prepared by the Standards Laboratory, Colindale. Where cells showed an adenovirus-type CPE, the culture fluid was tested for presence of adenovirus group complement-fixing antigen. Further identification of serotype was made by specific neutralizing sera.

RESULTS

One hundred and sixty-eight viruses were isolated from the faecal specimens, 148 (88%) being enteroviruses and 20 (12%) adenoviruses. The types of virus isolated and the frequency of isolation in each cell system are shown in Table 1.

TABLE 1
SENSITIVITY OF CELL CULTURES TO VIRUSES ISOLATED

Virus type	No. isolated	Monkey kidney		Human kidney	
		No.	%	No.	%
Polio	8	8	100	7	88
Coxsackie A	17	17	100	7	41
Coxsackie B	7	7	100	4	57
Echo	114	105	92	62	54
Unidentified [a]	2	2	100	0	0
Total enterovirus	148	139	94	80	54
Adenovirus	20	3	15	20	100
Total viruses	168	142	85	100	60

[a] Producing typical enterovirus CPE but not neutralized by available enterovirus antisera.

All 20 adenoviruses were isolated in HEK cells and were of types 1, 5, 7, 12, 17, 18 and 31. Only 3 strains (all type 5) were isolated in MK cells. This reflects the well-recognized affinity of human adenovirus for human fetal tissues (Sohier et al., 1965). For enterovirus isolation, however, MK cells proved markedly more sensitive, accounting for 94%, nearly twice the frequency in HEK cell cultures. The range and frequency of enterovirus isolations in each cell system is shown in Table 2. The enteroviruses are represented by 21 types including 15 types of echoviruses. (The coxsackievirus B group is proportionally under-represented because during an outbreak due to coxsackievirus B2, MK cells were found to be highly susceptible to the particular strain and many faecal specimens over this period of time were cultured on MK cells only.)

It can be seen from Table 2 that the *spectrum* of sensitivity of MK and HEK cells is very similar. Four virus types—coxsackievirus B3 and echoviruses types 4, 12 and 15—were isolated in MK cells only but the numbers of isolations were small in each case and little significance can be attached to this feature.

TABLE 2

ENTEROVIRUS TYPES AND CELL SUSCEPTIBILITY

Virus type	No. isolated	Monkey kidney and human kidney	Monkey kidney only	Human kidney only
Polio				
2	4	4	0	0
3	4	3	1	0
Coxsackie A9	17	7	10	0
Coxsackie				
B3	1	0	1	0
B4	5	3	2	0
B5	1	1	0	0
Echo				
1	1	1	0	0
2	2	1	1	0
3	3	0	2	1
4	3	0	3	0
5	2	1	1	0
6	40	21	18	1
9	16	8	8	0
11	4	2	1	1
12	1	0	1	0
14	6	2	3	1
15	1	0	1	0
19	5	2	2	1
25	4	2	2	0
30	25	12	9	4
31	1	1	0	0
Unidentified	2	0	2	0
Tota l	148	71 (48 %)	68 (46 %)	9 (6 %)

TABLE 3

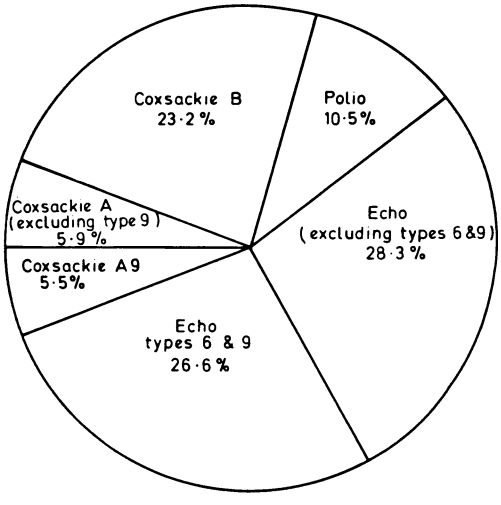
ENTEROVIRUSES ISOLATED IN SMALL NUMBERS AND CELL SUSCEPTIBILITY

Virus type	No. isolated	Monkey kidney	Human kidney
Polio			
2	4	4	4
3	4	4	3
Coxsackie			
B3	1	1	0
B4	5	5	3
B5	1	1	1
Echo			
1	1	1	1
2	2	2	1
3	3	2	1
4	3	3	0
5	2	2	1
11	4	3	3
12	1	1	0
14	6	5	3
15	1	1	0
19	5	4	3
25	4	4	2
31	1	1	1
Total	48	44 (92 %)	27 (56 %)

The *frequency* of isolation, however, was significantly different for the two cell systems. Nearly half the enteroviruses, representing 17 types, failed to grow in HEK cultures. It is known that a particular type or strain of enterovirus may demonstrate an altered cell affinity (Committee on Enterovirus, 1962; Suto et al., 1965a, 1965b). Such strains might unfairly bias a comparison should they occur in large numbers. To exclude this possibility comparison was made *excluding* strains for which more than 15 isolations were made (Table 3), and it can be seen that the results are essentially the same. It is clear, then, that the marked superiority of MK cells in this series is not based on one or two unrepresentative strains.

The significance of the poor performance of HEK cells in the isolation of coxsackievirus A9 and echoviruses types 6 and 9 may be more apparent when the prevalence of these three viruses in the population is considered. The numbers and frequency of enterovirus isolations in the United Kingdom during the three-year period 1967–69, as represented by reports made to the *Communicable Disease Report* of the Public Health Laboratory Service, are shown in the accompanying figure. It can be seen that echoviruses types 6 and 9 account for nearly half (45%) of the echovirus group isolations while coxsackievirus A9 isolations account for a similar proportion (48%) of the coxsackievirus A group.

ENTEROVIRUS ISOLATIONS REPORTED IN THE UNITED KINGDOM, 1967–69 [a]



[a] Frequencies based on a total of 7945 enterovirus isolations

Taken together, these three virus types account for one-third (32%) of the enterovirus isolations reported.

The rapidity with which a virus infection declares itself in cell culture is a variable that must be taken into account when assessing the value of a system for primary isolation from clinical material. A comparison of the time (in days) taken for a CPE to be clearly detected (approximately 25% of cells affected) is shown in Table 4. It can be seen that a CPE was detected marginally later in HEK cells (which were incubated at a lower temperature than MK cells) infected with poliovirus, coxsackievirus A9 and echoviruses. With coxsackieviruses of the B group, however, the detection of a CPE was significantly more rapid in MK cells, a finding which accords with the opinion that MK cells provide cultures of choice for rapid isolation of viruses of this group (Hambling & O'Neill, 1967).

TABLE 4

TIME (IN DAYS) TO PRODUCTION OF THE CYTOPATHIC EFFECT IN TISSUE CULTURE [a]

Virus type	Human embryo kidney		Monkey kidney	
	Mean	Range	Mean	Range
Polio	4.4	2–5	3.8	3–4
Coxsackie A9	5.4	2–11	4.4	3–7
Coxsackie B	13.8	7–24	5.3	3–7
Echo	6.7	3–19	6.2	2–17

[a] 56 strains.

DISCUSSION

Several studies have been reported concerning the comparative susceptibility of various cell systems for the *primary isolation* of viruses from faecal material (Kelly & Saunderson, 1962; Pal et al., 1963; Hambling & Davies, 1965; Lee et al., 1965). Using the term "enteric viruses" to denote both enteroviruses and adenoviruses, Lee et al. reported the comparative susceptibility of various cell systems including MK cell and HEK cell cultures. These workers considered HEK cells to be the best available cultures for the isolation of enteric viruses.

The results we report here also are concerned with a comparison of MK and HEK cells for the primary isolation of enteric viruses. Like the American workers, we found HEK cells much superior to MK cells for the isolation of adenoviruses, which constituted 12% of isolates in our series and 25% in theirs. When, however, the *enterovirus* isolations are considered, a marked difference between the two series emerges. Lee and his colleagues were able to isolate 82% of their enteroviruses in HEK cultures against 57% in MK cells and in addition found the spectrum of sensitivity of the former cells to be significantly wider. In our series the findings were almost the reverse. We were able to isolate only 54% of the enteroviruses in HEK cells against 94% in MK cells and the latter cells, in our hands, also had the wider virus spectrum.

The difference between our findings and those reported by the American workers are due in part to the different proportions of coxsackie A viruses found in the two studies. In our series this group was represented by a single virus type, coxsackievirus A9, the only serotype readily and consistently isolated in MK cells (Melnick, 1962) and thus behaving more like a group B virus. The American series, on the other hand, contained seven serotypes, namely coxsackieviruses A9, 13, 15, 17, 18, 20 and 24, which accounted for over a quarter (26%) of their enterovirus isolations. Apart from coxsackievirus A9, these viruses were isolated almost exclusively in HEK cells. The susceptibility of a cell system to a wide range of coxsackie A viruses is

certainly a highly commendable feature. The importance, however, of this facility will in general be proportional to the prevalence of these viruses in the population sampled—and this is likely to vary from time to time and place to place (Gelfand, 1961). It would appear relevant, therefore, to note that in the United Kingdom during the three-year period covered by this report, coxsackie A viruses (excluding coxsackievirus A9) accounted for less than 6% of the enteroviruses isolated (see the figure).

It is with the much larger portion represented by the remaining enteroviruses that disagreement is clearly seen. Whereas Lee and his colleagues found HEK cells more sensitive than MK cells for poliovirus, coxsackievirus B and echoviruses, we were able to isolate almost twice as many of these viruses in MK cultures as in HEK cells. The explanation for these conflicting findings is not clear. The quality of the cells, the composition of the medium, the techniques used and the particular host characteristics of the virus strain isolated may each play a part in determining the success or failure of a particular cell system. At the moment we do not have sufficient information to allow us to attempt to relate these features to the different findings of the two studies, although strain differences would seem unlikely to be significant in view of the numbers involved.

For the present we must conclude that HEK cells have been found much inferior to rhesus MK cells for the primary isolation of enteric viruses in the specimens submitted to us. We cannot, therefore, support the unqualified claim that HEK cells provide the culture of choice for the primary isolation of enteric viruses. The usefulness of these cells would appear to be directly proportional to the prevalence of adenoviruses and coxsackie A viruses in the population sampled.

RÉSUMÉ

ÉTUDE COMPARATIVE DE DEUX SYSTÈMES DE CULTURE CELLULAIRE POUR L'ISOLEMENT PRIMAIRE DES ENTÉROVIRUS

On a comparé la sensibilité respective de deux types de culture cellulaire, rein embryonnaire humain (HEK) et rein de singe rhésus (MK), aux virus présents dans des échantillons de selles.

Au total, 168 virus ont été isolés. Les cellules HEK se sont montrées beaucoup plus réceptives aux adénovirus, permettant d'identifier 20 souches de ce type, alors qu'on n'a recueilli que 3 souches sur cellules MK. En revanche, il est apparu que les cellules MK étaient davantage propices à l'isolement des entérovirus; 139 souches (94%) sur un total de 148 ont été isolées sur ce milieu et 80 seulement (54%) sur cellules HEK. La plus grande sensibilité des cellules MK s'est manifestée à l'égard de l'ensemble de la gamme des entérovirus et n'a pas été due à l'isolement de l'une ou l'autre souche non caractéristique. Sous le rapport de la vitesse d'infection des cellules de chaque système, on n'a relevé aucune différence nette dans la production de l'effet pathogène par les poliovirus, le coxsackievirus A9 et les échovirus, mais l'infection des cellules par les coxsackievirus B a été décelée plus précocement dans le système MK.

Des travaux précédents ont fait ressortir la supériorité des cellules HEK pour l'isolement primaire des entérovirus. Les raisons de la discordance entre ces résultats et ceux décrits dans le présent article sont discutées. Selon les auteurs, l'efficacité du système HEK est directement fonction de la prévalence des adénovirus et des coxsackievirus A dans l'échantillon de population examiné.

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