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Faecal adenoviruses from Glasgow babies

Studies on culture and identity

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SUMMARY

Attempts were made to isolate viruses from babies' stools that contained adenoviruses detected by electron microscopy. One hundred and fifty-nine specimens from 71 children were studied and adenoviruses of established serotypes were isolated from 81 stools. Serial stool samples containing adenovirus particles were obtained from 35 children, and prolonged shedding of recognized serotypes was common. Simultaneous and sequential infections by different serotypes were also observed. Thirty-six children shed adenoviruses that could not be isolated using cell cultures normally used to detect adenoviruses, and nine of these children also shed adenoviruses of established serotypes. Passage in Chang conjunctival cell cultures allowed characterization of fastidious adenoviruses from 14 children as members of a previously unrecognized serotype.

INTRODUCTION

The use of electron microscopy (EM) in virology has focussed attention on rotaviruses, adenoviruses, coronavirus-like particles, caliciviruses, astroviruses and other small round viruses as possible enteric pathogens of young children (Holmes, 1979; Madeley, 1979). Rotaviruses are widely accepted as a cause of infantile diarrhoea throughout the world (Kapikian *et al.* 1979) but the role of adenoviruses in causing diarrhoea requires further investigation.

Established serotypes of adenovirus have been isolated from the stools of children for many years. Types 1 and 2 are especially common and faecal shedding may persist for many months (Bell *et al.* 1961; Brandt *et al.* 1969; Fox *et al.* 1969). Prospective studies to investigate a possible association between the shedding of common adenovirus serotypes and diarrhoea have been inconclusive (Moffet,

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Shulenberger & Burkholder, 1968; Yow *et al.* 1970). However, not all adenovirus strains seen in stools by EM can be isolated in cell cultures (Bryden *et al.* 1975; Schoub *et al.* 1975; White & Stancliffe, 1975) and there have been reports that these fastidious adenoviruses may be associated with outbreaks of diarrhoea (Flewett *et al.* 1975; Whitelaw, Davis & Parry, 1977; Richmond *et al.* 1979). Moreover, adenoviruses that could not be cultured were detected by EM more frequently in stools from children with diarrhoea than in those from control children (Brandt *et al.* 1979).

We have previously reported (Kidd & Madeley, 1981) that some fastidious adenoviruses cause cytopathic effects (CPE) in Chang conjunctival cells (Wong & Kilbourne, 1961), which are probably derived from HeLa cells (Nelson-Rees & Flandermeyer, 1976). This CPE could be neutralized by rabbit antisera raised against one fastidious strain, to titres of 640 or greater. The antisera have been found to lack neutralizing antibodies to the 37 previously described serotypes (de Jong *et al.* 1981). This is evidence that fastidious adenoviruses constitute at least one new serotype.

This report describes a two-year study into the shedding of faecal adenoviruses by children under 3 years of age in Glasgow. We describe observations on the serotypes excreted, the duration of excretion, and, in particular, some features of the shedding of adenoviruses that are difficult to culture.

PATIENTS AND METHODS

Stool specimens. Stools from children in Glasgow were received for examination by EM between October 1975 and January 1978. Except where otherwise stated, the specimens were found to contain adenoviruses by EM before being tested in cell culture.

The stools were from two studies: (*a*) children admitted to hospital with diarrhoea (Madeley *et al.* 1977), and (*b*) children from whom several stool samples were taken in a study of viruses shed in a deprived urban community (Scott *et al.* 1979). In the second group, serial stool specimens were taken regularly from children at home and more frequently during one or more periods in hospital.

Electron microscopy. The preparation of stool extracts (clarified stool suspensions) and examination by EM have been described previously (Madeley *et al.* 1977).

Virus isolation and identification. Except where otherwise stated, stool extracts were tested blind in culture, using day-book numbers rather than names to identify them. Thus different stools from the same child were studied separately and any isolates were typed independently. Test tube cultures of primary human amnion and/or human embryo kidney (HEK) cells maintained in Eagle's MEM with 0·5% fetal calf serum and antibiotics were inoculated with 0·1 ml unfiltered stool extract and incubated stationary at 37 °C. Those specimens which caused CPE were passaged for confirmation before identification of the agent(s) was attempted by neutralization tests (NT). NTs for adenoviruses were performed according to standard procedures (Rowe, Hartley & Huebner, 1958), using type-specific rabbit

Table 1. *Number of stool specimens per patient, positive for adenovirus particles by EM*

No. of stools per patient	No. of patients	Total no. of stools
1	36	36
2	13	26
3	10	30
4	6	24
5	2	10
6	0	0
7	2	14
8	1	8
9	0	0
10	0	0
11	1	11
Total	71	159

antisera kindly supplied by Dr E. J. Bell, Enterovirus Reference Laboratory (Scotland). Cross reactions between types 12 and 31 have been recorded (Rafajko, 1966; Brandt *et al.* 1969) and for this reason all Ad 12-and Ad 31-related strains are referred to as Ad 12–31. Some subgroup A adenoviruses passaged poorly in human amnion and HEK cells after isolation. These viruses were propagated and typed using Chang conjunctival cells (Kidd & Madeley, 1981).

The preparation of neutralizing antisera in rabbits to the fastidious adenovirus strain from patient no. 25 and the culture of fastidious adenoviruses in Chang conjunctival cells have already been described (Kidd & Madeley, 1981). Titrations of these antisera against fastidious strains by NT were performed using undiluted virus. The tests were read at 5–7 days when approximately 50% of the monolayer in the virus controls showed CPE. The titre was taken as the highest serum dilution to inhibit CPE completely.

Terminology

Adenovirus strains were described as fastidious if they could not be passaged serially in HEK cells with CPE. All such strains were tested in HEK cell cultures over 4 weeks with at least one blind passage.

RESULTS

During the two-year study, 159 stools were found to contain adenoviruses by EM. The specimens were from 71 children and the numbers of children from whom more than one EM positive specimen was obtained are shown in Table 1. Fast growing enteroviruses, most of which gave CPE in 24 h, were isolated from 14 of the specimens and no further attempt was made to isolate the adenovirus. Established adenovirus serotypes were isolated from 81 stools from 40 children

Table 2. *Serotypes of adenovirus isolated from* 159 *stool specimens known to contain adenovirus particles by EM*

Adenovirus serotype	No. of isolates	No. of patients from whom each type was obtained
1	22	12
2	28	15
1+2*	1	1
3	1	1
5	3	3
7	1	1
9	1	1
2+9*	2	1
14–16†	3	1
17	2	2
18	3	3
12–31†	14	10
f	64	36
ent	14	8
Total	159	71

* Simultaneous infection by 2 serotypes.

† Adenovirus strains not resolved as one or other serotype by NT.

f, fastidious adenoviruses, i.e. those which were not isolated using HEK cells; ent, enterovirus, presumed or typed.

(Table 2). Fastidious adenoviruses, i.e. those causing little or no CPE in HEK cells and/or which could not be typed satisfactorily using standard antisera, were found in 64 specimens from 36 children (Table 2).

Established adenovirus serotypes. Serial stool samples containing adenovirus particles were obtained from 35 children and details of the adenovirus types found are given in Table 3. The longest interval between stools in which the same serotype was found was 231 days (Ad2 from patient no. 12). Seven children shed more than one established adenovirus serotype (patients no. 5, 6, 7, 8, 11, 14 and 28). Patients no. 5, 7 and 14 each shed three different serotypes within a period of 150, 32 and 123 days respectively. In two of these cases, adenoviruses of more than one established serotype were isolated from the same stool. Patient no. 5 shed both Ad2 and Ad9 in each of two stools taken 10 days apart. In the case of patient no. 7, the shedding of both Ad1 and Ad2 in the same stool marked a changeover from excretion of predominantly Ad2 to excretion of Ad1.

Thirteen children shed adenoviruses of subgroup A (Ad12, Ad 18, Ad 31; Green *et al.* 1979). Identification of the three Ad18 isolates was unequivocal but the other subgroup A strains were neutralized to some extent by antisera to both Ad12 and Ad31 prototypes. Adenoviruses related to Ad12 and Ad31 were isolated from two stools taken 180 days apart from patient no. 28. This may represent prolonged shedding of one strain, or sequential infection by two different strains related by different degrees to Ad12 and Ad31.

Table 3. *Adenoviruses in stool specimens from 35 children from whom multiple specimens, EM positive for adenovirus, were obtained*

Patient	Results of EM examination of stools: Total no. examined	Results of EM examination of stools: No. adenovirus positive	Adenoviruses found (day of recognizate)	Minimum period of shedding (days): overall (fastidious)
U	18	2	*f*(1), *f*(2).	1 (1)
V	10	2	Ad1(1), *f*(2).	1 (< 1)
3	6	4	*f*(1), *f*(4), *f*(5), *f*(7).	6 (6)
4	2	2	Ad2(1). Ad2(4).	3
5	71	11	Ad12–31(1), Ad2(60), Ad2(62), Ad2(68), Ad2 + 9(151) Ad2 + 9(161), Ad2(170), {Ad2 / *f*}(186), E3(200), Ad2(210).	209 (< 1)
6	40	7	*f*(1), Ad1(87), {Ad1 / Ad1}(91), Ad1(92), Ad9(134), ent(163).	162 (< 1)
7	51	8	{Ad2 / Ad2}(1), Ad2(4), Ad17(28), Ad1 + 2(36), Ad1(80). Ad1(144), Ad1(218).	217
8	32	4	Ad5(1), Ad14–16(16), Ad14–16(25), Ad14–16(86).	85
9	43	4	*f*(1), *f*(7), *f*(9), Ad7(105).	104 (8)
11	37	2	Ad2(1), Ad18(238).	237
12	34	4	*f*(1), Ad2(8), ent(218), Ad2(239).	238 (< 1)
13	2	2	Ad1(1), Ad1(2).	1
14	60	5	Ad17(1), Ad12–31(4), E11(96), ent(106), Ad2(124).	123
20	3	3	*f*(1), *f*(3), *f*(6).	5 (5)
23	4	3	P2(1), ent(2), ent(3).	2
24	3	2	Ad1(1), Ad1(9).	8
25	40	7	*f*(1), *f*(3), *f*(6), *f*(7), *f*(9), Ad1(141), Ad1(148).	147 (8)
27	10	2	*f*(1), Ad12–31(268).	267 (< 1)
28	43	3	Ad12–31(1), Ad12–31(181), Ad1(202).	201
29	4	3	*f*(1), {*f* / *f*}(3).	2 (2)
31	2	2	Ad2(1), Ad2(2).	1
34	5	4	*f*(1), *f*(3), *f*(4), *f*(5).	4 (4)
35	2	2	Ad12–31(1), Ad12–31(2).	1
36	3	3	{*f* / *f*}(1), *f*(5).	4 (4)
37	3	3	Ad1(1), {Ad1 / Ad1}(3).	2
38	7	5	*f*(1), *f*(2), *f*(3), *f*(4), *f*(5).	4 (4)
39	39	2	Ad12–31(1), Ad12–31(5).	4
40	4	3	*f*(1), Ad12–31(2), Ad12–31(5).	4 (< 1)
41	3	3	Ad2(1), Ad2(2), *f*(3).	2 (< 1)
43	3	3	{*f* / *f*}(1), *f*(2)	1 (1)
46	6	3	Ad2(1), {Ad2 / Ad2}(2).	1
48	6	4	E13(1), ent(3), ent(4), ent(5).	4
52	7	2	*f*(1), *f*(2).	1 (1)
59	2	2	*f*(1), *f*(2).	1 (1)
68	2	2	{*f* / *f*}(1).	1 (< 1)

Ad, adenovirus; *f*, fastidious adenovirus, not isolated using HEK cells; ent, presumed enterovirus, untyped; P, poliovirus; E, echovirus.

Shedding of fastidious adenoviruses. Simultaneous excretion of more than one adenovirus serotype was found in this study. Consequently, there is no certainty that an adenovirus seen by EM was the one grown. The last column in Table 3 lists, for each child, the longest interval over which adenoviruses were detected by EM and, in brackets, that over which only fastidious (*f*) adenoviruses were found.

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Table 4. *Fastidious adenovirus strains that were passaged in Chang conjunctival cells*

Patient no.	Date of stool specimen	Passage history: No. of passages in Chang cells: Tried	Passage history: No. of passages in Chang cells: In which CPE developed	Passage history: Passage no. of fluid tested by NT	Titre to which isolate was neutralized by anti-fastidious adenovirus antiserum
3	6. xii. 75	2	2	1	20*
9	15. xii. 75	4	3	1	⩾ 640
20	1. vii. 76	6	6	2	160
21	5. vii. 76	4	2	1	⩾ 640
29	26 vii. 76	5	5	2	⩾ 640
38	18 i. 77	2	2	1	20*
41	5. ii. 77	2	2	1	20*
62	8. x. 77	3	2	1	⩾ 640
64	17. x. 77	9	9	4	⩾ 640
68	21. xi. 77	5	4	2	⩾ 640
	21. xi. 77	5	4	3	⩾ 640
69	28. xi. 77	9	9	4	⩾ 640
5	2. viii. 76	One passage organ cultures and one passage Chang cells			320
25	25. vii. 76				⩾ 640
30	10. viii. 76				⩾ 640

* Tested at antiserum dilution of 1 in 20 only.

At least 36 children shed fastidious adenoviruses, and nine of these patients shed fastidious adenoviruses and known serotypes at different times (Table 3). Twelve children shed fastidious adenoviruses on more than one day and these viruses were detected in stools taken over 4–8 days from seven children. No established adenovirus serotypes were isolated during the observed periods of fastidious adenovirus excretion, though in some cases not all of the stools passed in this period were examined by EM. Patient no. 5 shed Ad2 and fastidious adenoviruses on the same day. It is not known which strain was shed first, but the fastidious strain was distinct from the established serotypes (see below). No seasonal variation in the excretion of fastidious adenoviruses was observed.

Identification of fastidious strains. Several specimens from children described in this paper were completely used before the advantages of Chang conjunctival cell cultures over HEK cultures were recognized. However, fastidious adenoviruses from 11 of the children caused CPE in Chang cells incubated at 33 °C and were antigenically related by NT using the antisera raised against fastidious adenoviruses from patient no. 25 (Table 4). Fastidious adenoviruses from three other children (patient no. 5 (the strain described above), patient no. 25 (virus homologous to the antiserum) and patient no. 30) each caused CPE in Chang cells after both one and two passages in organ cultures of fetal human intestine (Kidd & Madeley, 1981). The organ culture isolates were also neutralized by antiserum to the presumptive new serotype.

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Table 5. *Number of children excreting fastidious adenoviruses by age*

Age (months)	1–2	3–4	5–6	7–8	9–10	11–12	> 12	Total
No. of children shedding fastidious adenoviruses	13	8	5	0	4	3	3*	36
No. of children known to shed presumptive new serotype	5	3	2	0	2	1	1†	14

* Shedding at 13, 25 and 36 months of age.
† Shedding at 13 months of age.

Amounts of adenovirus seen by EM. There have been reports that adenoviruses difficult to culture are present in stools in greater numbers than those that are isolated easily (Flewett, 1977; Brandt *et al.* 1979). Our observations support this view but difficulties in making each EM preparation comparable make this difficult to prove.

Age at shedding of fastidious adenoviruses. Table 5 shows the ages of the 36 children at the time that they shed fastidious adenoviruses. These ranged from one month to three years. Of the 14 strains known to belong to a new serotype, all were shed by children between the ages of one and 13 months.

Fastidious adenoviruses and illness. Thirty-two of the 36 children who shed fastidious adenoviruses had watery or frequent stools and/or vomiting within one day of excreting these viruses. All the 14 children known to have shed adenoviruses of the previously unrecognized serotype had diarrhoea on the day before, on the same day, or on the day after shedding. The apparent association of fastidious adenovirus shedding with diarrhoea is equivocal, because there is still uncertainty about the duration of excretion of these viruses.

Intensive study of stools from three children. Fastidious adenoviruses may be more infectious for cell cultures when present in small numbers. To investigate this, the stool specimens collected from three children over an 8–10 month period were tested in Chang cell cultures, irrespective of whether adenovirus particles were detected by EM. Table 6 lists all the viruses found in the stools of these three babies and gives as complete a profile as possible of the stool viral flora over the period of study. Thirty-nine specimens from patient no. 6 and 37 specimens from patient no. 25 were tested. Both children shed fastidious adenoviruses, but these were only detected by culture from stools known to contain adenovirus particles by EM. In contrast, both children shed Ad1 several weeks after shedding fastidious strains, and sometimes in amounts too low to be detected by EM.

Only four of 31 stools tested from patient no. 8 contained adenovirus particles by EM, whereas 18 were positive by culture. This child was infected by four different established serotypes over a 6-month period, and each serotype belonged to a different subgroup of human adenoviruses (Green *et al.* 1979). The relative ease by which these established adenovirus serotypes were recovered from stools may have hidden the presence of any fastidious strains, but there was no evidence that

Table 6. *Culture results of 107 stool specimens from 3 children, using Chang conjunctival cells. No entry in the table indicates that no virus was detected*

Patient no. 6			Patient no. 8			Patient no. 25		
Date of stool	Virus by EM	Virus by Culture	Date of stool	Virus by EM	Virus by Culture	Date of stool	Virus by EM	Virus by Culture
15. xii. 75	—	—	18. i. 76	—	—	15. vi. 76	—	—
16. xii. 75	—	—	20. i. 76	—	—	19. vi. 76	—	—
18. xii. 75	—	—	21. i. 76	SRV	—	29. vi. 76	—	—
12. ii. 76	Ad	*	22. i. 76	—	—	30. vi. 76	—	—
13. ii. 76	SRV	—	28. i. 76	SRV	Ad12–31	1. vii. 76	—	—
13. ii. 76	SRV	—	15. iii. 76	Ad	Ad5	2. vii. 76	Rota	—
16. ii. 76	—	—	16. iii. 76	Ad	Ad5	3. vii. 76	Rota	—
17. ii. 76	—	—	23. iii. 76	—	Ad5	5. vii. 76	—	—
4. iii. 76	—	—	31. iii. 76	—	Ad5	12. vii. 76	Ast	—
16. iii. 76	—	—	6. iv. 76	Ast	Ad5	13. vii. 76	Ast	—
22. iii. 76	Ast	—	12. iv. 76	SRV	Ad5	14. vii. 76	Ast	—
24. iii. 76	SRV	—	21. iv. 76	—	Ad5	15. vii. 76	—	—
29. iii. 76	—	—	27. iv. 76	—	—	17. vii. 76	—	—
16. iv. 76	—	—	4. v. 76	—	—	19. vii. 76	Ad	—
16. iv. 76	—	—	11. v. 76	—	Ad5	21. vii. 76	Ad	†
18. iv. 76	—	—	12. v. 76	—	Ad5	25. vii. 76	Ad	—
18. iv. 76	—	—	17. v. 76	SRV	—	2. viii. 76	—	—
20. iv. 76	SRV	—	24. v. 76	—	Ad14–16	9. viii. 76	—	—
27. iv. 76	—	—	31. v. 76	Ad	Ad14–16	17. viii. 76	—	—
4. v. 76	—	Ad1	9. vi. 76	Ad	Ad14–16	2. ix. 76	—	-
7. v. 76	—	P1	21. vi. 76	—	Ad5	16. ix. 76	—	—
8. v. 76	Ad	Ad1	7. vii. 76	—	—	29. ix. 76	—	—
10. v. 76	—	Ad1	15. vii. 76	—	—	11. x. 76	SRV	—
12. v. 76	Ad	Ad1	22. vii. 76	—	—	18. x. 76	—	—
12. v. 76	Ad	Ad1	26. vii. 76	SRV	Ad5	8. xi. 76	—	ent
13. v. 76	Ad	Ad1	2. viii. 76	—	Ad9	22. xi. 76	—	B5
24. vi. 76	Ad	Ad9‡	9. viii. 76	Ad	Ad14–16	6. xii. 76	Ad	ent
14. vii. 76	—	Ad1	18. viii. 76	—	—	13. xii. 76	Ad	Ad1
23. vii. 76	Ad	Ad1	25. viii. 76	—	—	21. xii. 76	—	—
28. vii. 76	—	—	4. x. 76	—	Ad5	29. xii. 76	—	Ad1
29. vii. 76	—	—	11. x. 76	—	—	5. i. 77	—	—
18. viii. 76	—	—	—	—	—	10. i. 77	—	—
31. viii. 76	—	—	—	—	—	18. i. 77	—	—
31. viii. 76	—	Ad1	—	—	—	24. i. 77	—	—
20. x. 76	—	—	—	—	—	17. ii. 77	—	—
21. x. 76	—	Ad1	—	—	—	24. ii. 77	—	—
22. x. 76	—	Ad1	—	—	—	28. ii. 77	—	—
25. x. 76	—	—	—	—	—	—	—	—
26. x. 76	—	—	—	—	—	—	—	—

* CPE over one passage only; untyped.

† CPE over two passages only; untyped. A specimen from this patient was also tested in organ cultures of foetal human intestine (see Table 4).

‡ Ad9 isolated using HEK cells, not Chang cells.

Ad, adenovirus; Ast, astrovirus; Rota, rotavirus; SRV, small round virus; ent, presumed enterovirus, untyped; B, coxsackie B virus; P, poliovirus.

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the new serotype could be isolated from stools that were negative for adenoviruses by EM.

DISCUSSION

Adenoviruses in faeces could be the result of carriage through the gut from a respiratory infection, or they could result from replication in cells of the intestine. Common established adenovirus serotypes can replicate in the adult gut when administered orally by capsule to by-pass the respiratory tract (Couch *et al.* 1963; Schwartz, Togo & Hornick, 1974) and it seems likely that natural replication in the gut of children must occur to produce the large qualtities of adenovirus particles often seen in stools by EM. Why the infection is not eliminated in many cases is unknown. Adenoviruses can remain, apparently dormant, in tonsillar tissue (Strohl & Schlesinger, 1965) and they may similarly colonize the Peyer's patches with production of infectious progeny at intervals. Excretion over several weeks could also be due to infection progressing from one part of the gut to another. The relative importance of local immunity, mediated through antibody and/or cells, in limiting such infections is unknown. This may depend on the antigenic and/or invasive properties of the adenovirus strain.

Our results show that adenoviruses of different established serotypes may be shed by one child in succession or simultaneously over a period of days. Dual and triple infections are probably common in children, and prolonged and overlapping infection by different adenovirus serotypes could provide an opportunity for new strains to emerge by recombination.

Our results and those of others have shown that neither cell culture methods nor EM will allow the detection of all adenoviruses in stools, and both must be used for maximum sensitivity. Immunofluorescence is also useful, but in our experience not all fastidious adenoviruses in stools can be detected by this method (using either HEK or KB cells fixed up to 10 days after inoculation). The most recent methods for detecting adenoviruses are radioimmunoassay (Halonen *et al.* 1980) and enzyme linked immunosorbent assay (ELISA) (Johansson *et al.* 1980). ELISA has been used to detect both group antigens and antigens peculiar to fastidious adenoviruses in stools taken from six children studied in this paper (nos. 3, 9, 12, 20, 25 and 29). This antigenic relationship was later confirmed by passage and neutralization, with the exception of the fastidious adenovirus from patient no. 12, which did not passage in Chang cells.

It remains to be seen whether all fastidious adenoviruses are related antigenically. Antibodies capable of neutralizing the fastidious adenovirus from patient no. 69 were present in 42% of sera taken from 67 children less than 6 years of age in London (Kidd *et al.* 1981). The study indicated that some 50% of children may experience infection by this or a related serotype by three years of age. Moreover, we have detected stains of this serotype in stools from South Africa and Malaysia (A. H. Kidd, unpublished results) and this suggests a wide distribution. Wadell *et al.* (1980) found that 7 Glasgow strains (including those from patients no. 12, 25 and 29) were indistinguishable from fastidious adenoviruses from Scandinavia and Italy using restriction endonuclease analysis. However, there appear to be

other fastidious adenoviruses which are closely related to the Glasgow strains by neutralization and haemagglutination-inhibition tests but not by DNA restriction analysis (Uhnoo *et al.* 1981; de Jong *et al.* 1981).

The word 'enteric' has been used to describe adenoviruses which do not replicate in cell cultures commonly used in the diagnostic laboratory (Jacobsson, Johansson & Wadell, 1979). However, some high-numbered adenovirus serotypes are isolated almost exclusively from faecal specimens (Rosen, Hovis & Bell, 1962) and might also be described as enteric strains. That fastidious adenoviruses have not been isolated from respiratory specimens may be more a result of the shortcomings in current cell culture systems than an exclusive predilection of the virus for cells of the intestine. We prefer to use the word 'fastidious' until the serotype(s) involved are numbered officially.

Common established adenovirus serotypes were shed over several weeks in this study, but excretion of fastidious strains was not observed for longer than eight days. However, the duration of excretion may have been longer if non-cytopathogenic strains were shed in low numbers or were outgrown by established serotypes. Outbreaks of shedding of unidentified adenoviruses described in the literature suggest that infection may be acute and often severe (Flewett *et al.* 1975; Whitelaw, Davis & Parry, 1977; Richmond *et al.* 1979). Moreover, our observations and those of others indicate that these elusive adenovirus strains may be shed in very large numbers. Their successful transmission may rely on shedding in large quantity over a short period rather than shedding in small quantity over a longer interval.

ADDENDUM

After submission of this paper, Takiff, Straus & Garon (*Lancet* 1981, ii, 832–834) reported the propagation of previously non-cultivable viruses in 293 cells. We can confirm the usefulness of 293 cells in serial passage of some, but not all, fastidious adenovirus strains. Although such viruses appear to retain their unique identity by serum neutralization we must express caution in passaging reference strains in a cell line which has endogenous DNA sequences of another serotype.

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