ASSOCIATION BETWEEN DIARRHOEA AND ADENOVIRUS TYPE 7

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During October, 1958, the Health Department became aware of an increase in notifications of cases of diarrhoea in Newbiggin. Cases which were notified had stools examined routinely for bacterial pathogens, and it was noted that less than half of the cases were excreting *Shigella sonnei*, while the rest were negative for bacterial pathogens. The possibility of a virus cause was considered. Ramos-Alvarez and Sabin (1958) found the E.C.H.O. group of viruses more common in children with diarrhoea, as did Sommerville (1958) in Scotland. Neither group of workers found an increase in adenovirus isolation associated with diarrhoea. Kjellén *et al.* (1957a, 1957b), however, reported a number of cases with respiratory infection caused by type 3 adenovirus which also suffered from diarrhoea. It was considered of sufficient interest to describe this outbreak of diarrhoea in which adenovirus type 7 was isolated from a number of cases.

The Outbreak

The outbreak at Newbiggin started at the beginning of October, and from October 1 to December 31, 1958, 99 cases were notified (see Chart). Of these, only 40

Distribution of the Newbiggin cases. White columns represent total number of cases notified. Black columns represent confirmed cases of *Sonne* dysentery.

were confirmed as cases of *Sonne* dysentery. No bacterial pathogens were isolated from the remaining 59. Cases of diarrhoea also occurred in Ashington, a neighbouring town, and between October 1 and December 31, 68 cases were notified, of which 64 were confirmed bacteriologically as *Sh. sonnei*; in the remaining four no bacterial pathogens were incriminated.

Clinical Description.—The clinical picture of the cases in Newbiggin was that of an acute attack of abdominal pain, followed immediately by diarrhoea with a very watery stool. Associated with this were nausea or actual vomiting, fever, headache, sore throat, and a cough. One child in Ashington had enlarged cervical and iliac glands; unfortunately her stools were not examined for adenovirus, but they did contain *Sh. sonnei*. Cases varied in severity from those with only abdominal pain followed by diarrhoea, which lasted for one or two days, to the more severe cases which had the clinical appearance of "gastric influenza." In the Ashington cases the respiratory symptoms were of less significance. All age-groups were affected, and the cases were widely scattered in the town.

Methods

It was not until the outbreak had been in progress for a few weeks that something unusual was suspected and that stools were examined for viruses. Originally it was expected that if viruses were likely to be associated with diarrhoea they would be of either the E.C.H.O. or the Coxsackie group of enteroviruses (Ramos-Alvarez and Sabin, 1958; Sommerville, 1958). On first isolating an adenovirus it was decided that all cultures for attempted virus isolation would have to be kept for at least 28 days before being discarded as negative. A total of 31 faeces were examined. As most of the cases were in children seen in general practice it was impossible to take paired sera.

Virus isolations were attempted by methods similar to those described by Munro-Ashman *et al.* (1958). HeLa-cell cultures were grown in a medium containing 15% human serum; before inoculation the serum was removed from the cell culture by washing and replaced by a maintenance medium containing 5% horse serum. 0.2 ml. of a 10% suspension of faeces in Hanks's salt solution was inoculated into each of two tubes of HeLa-cell cultures. The cultures were examined daily for 28 days before being discarded as negative. It was usually necessary to pass material at least once during the 28 days.

When degeneration suspected of being due to an adenovirus occurred the culture tubes were frozen and thawed and the fluid was subjected to a complement-fixation test for the presence of adenovirus, using a known positive serum. Viruses found in this way were typed in HeLa-cell cultures containing 0.5 ml. of maintenance medium, to which was added 0.1 ml. of type-specific serum followed by 0.1 ml. of suspected virus under test. The dose of virus was adjusted to give complete degeneration in two to three days. The sera used for typing were types 1-8. They had been prepared in rabbits which had received a series of intravenous injections of supernatant fluids from HeLa-cell cultures infected with adenovirus serotype strains. As a control, the faeces of 50 normal children aged 5 years or under, from Newcastle, about 15 miles from Newbiggin, were examined in a similar way to that described for the isolation of adenovirus.

Results

Of the 31 faeces from cases of diarrhoea examined for the presence of adenovirus, 10 had adenovirus type 7 present. All were from children 5 years of age or under, with the exception of one isolated from an adult. These cases occurred in seven families. Of the 21 patients with no adenovirus in their faeces, 10 were over the age of 5 and 11 were 5 or less. A brief description is now given of the adenovirus-positive cases; all came from Newbiggin except Cases 5, 6, and 7, which came from Ashington.

Cases 1 and 2.—A brother and sister, aged 3 and 4 years respectively, were taken ill on October 28 with abdominal pain and diarrhoea. *Sh. sonnei* was not isolated from their stools and there was no history of respiratory illness. A cousin of theirs aged 3 years was taken ill a week previously with abdominal pain and diarrhoea. His stools were free from *Sh. sonnei*, but unfortunately they were not available for virological studies.

Case 3.—A girl aged 2 years had an upper respiratory infection on October 26 followed by vomiting and diarrhoea. Her brother, aged 3 years, had sore throat, diarrhoea, and vomiting, which started on October 24. *Sh. sonnei* was present in both stools, but the boy's stools were not examined for adenovirus.

Case 4.—A girl aged 3 years was taken ill on November 23 with sore eyes, cough, and coryza. There was slight looseness of stools. *Sh. sonnei* was also present. Her sister, aged 6 years, had only diarrhoea, which began on November 29; *Sh. sonnei* was isolated, but no adenovirus.

Cases 5, 6, and 7.—A girl aged 5 years was the first in this family to go sick, on November 8 with abdominal pain; severe diarrhoea and a persistent hard cough developed at the same time. Her twin brother became ill on November 14 and was very much worse than his sister. He had sore throat, cough, and high fever. There was a bilateral conjunctivitis with photophobia. Abdominal pains and diarrhoea were present. Their mother became ill with diarrhoea on November 23 but had had some abdominal pains during the previous week. All three had adenovirus type 7 in their stools, but only the twins had *Sh. sonnei*.

Case 8.—This patient was a child aged 1 year whose illness began on November 19. At the onset he was feverish and had a heavy cold; vomiting and diarrhoea were present. *Sh. sonnei* was found in the stool as well as adenovirus. The father gave a history of becoming ill on November 10 with a severe sore throat and a very heavy cold. He had diarrhoea lasting one week. No faeces were available from him. No adenovirus was isolated from six older children of the family, but *Sh. sonnei* was found in two of them. All had abdominal pain and nausea, with a rise in temperature.

Case 9.—A child aged 5 years was taken ill on January 11, 1959, with vomiting and diarrhoea all night. A specimen collected on January 16 produced both *Sh. sonnei* and adenovirus type 7. On January 19 the child developed mouth ulcers, earache, sore throat, and backache and had loose motions. Three days earlier the brother, aged 8 years, became ill with nausea, diarrhoea, and vomiting but no respiratory symptoms. At this time the father, aged 31, returned from work complaining of abdominal pain and diarrhoea. This improved and he returned to work two days later, but on January 23 he reported sick again, with headache, aches and pains, sore throat, and some vomiting. The mother, aged 28, was taken ill on January 19 with headache, giddiness, aches and pains, and a temperature of 101° F. (38.3° C.). She vomited once, and had abdominal pain but no diarrhoea. On January 23 she developed a sore throat and had a head cold during that week-end. *Sh. sonnei* was isolated from the stools of all members of this family.

Case 10.—After the main outbreak, one further case of adenovirus type 7 was isolated from the stool of a baby,

aged 4 months, who had a history of vomiting and diarrhoea but no respiratory infection. *Sh. sonnei* was not isolated from this stool.

In Cases 3–9 there was either a clear history of respiratory infections or of a close association. In only one case out of 22 in which adenovirus type 7 was not isolated was there a history of respiratory illness, and this was in a woman aged 60 who had a mild coryza. Table I summarizes details of these patients and Table II summarizes all 31 cases.

TABLE I.—Patients From Whom Adenovirus Type 7 was Isolated

Case No.	Age in Years	Respiratory Signs	Diarrhoea and Vomiting	<i>Sh. sonnei</i> Isolated	Days before Culture Proved Positive
1	3	—	+	—	7
2	4	—	+	—	11
3	2	+	+	+	9
4	3	+	+	+	13
5	5	+	+	+	11
6	5	+	+	+	9
7	Adult	—	+	—	20
8	1	+	+	+	19
9	5	+	+	+	7
10	4/12	—	+	—	12

Of the 31 cases, 19 were found to have *Sh. sonnei* in stools. — = Absence of symptoms or of *Sh. sonnei*. + = Symptoms or *Sh. sonnei* present.

TABLE II

Description	No.	Percentage
Cases with <i>Sh. sonnei</i> only	10	32
" " adenovirus only	3	10
" " <i>Sh. sonnei</i> and adenovirus	6	19
Total with <i>Sh. sonnei</i>	16	52
" " adenovirus	9	29
Cases which were negative	12	39
Total cases examined	31	100
Cases over 5 years old examined	11	36
No. positive	1	9
		(of over 5 years of age)
Cases under 5 years old examined	20	64
No. positive	9	45
		(of under 5 years of age)

TABLE III.—Children Under the Age of 5 Years

	No. Examined	No. with Adenovirus
Children with diarrhoea and <i>Sh. sonnei</i>	20	0
" " " " no bacterial	20	0
Normal children	50	1 (type 6)

Control Results.—The stools from 20 children aged 0–5 years with Sonne dysentery were examined on HeLa cells for the presence of adenoviruses, with negative results. These stools were collected from a neighbouring town and each case represented a separate Sonne dysentery incident. Fifty stools of normal children were examined from the same area and one adenovirus type 6 was found. Twenty children, all under the age of 5, who had diarrhoea but in whom no bacterial cause was found, were examined for the presence of adenovirus, but all these were negative. The results are summarized in Table III.

Discussion

That adenovirus may cause diarrhoea was shown by Kjellén *et al.* (1957a, 1957b) in about 50% of children with an adenovirus type 3 infection. They demonstrated this adenovirus in stools and mesenteric glands of children suffering from mesenteric lymphadenitis. Cases

1 and 2 in the present series showed no respiratory symptoms, only diarrhoea and vomiting; in neither of these patients nor in their cousin, who developed a similar illness, was *Sh. sonnei* isolated. It seems unlikely that in all three cases *Sh. sonnei* should have been missed in the bacteriological examination of the stools, and it seems reasonable to assume that in these two cases adenovirus type 7 was closely associated with the illness. Of the remaining eight cases, four had definite respiratory symptoms, two had conjunctivitis, one had no definite symptoms, and in one, a child of 1 year, it was not clear whether he had a respiratory infection or not, but his father had a severe sore throat. A convalescent specimen of the father's serum was available and the titre of complement-fixing antibodies to adenovirus was 1/8. The complement-fixation test used was similar to that described by White *et al.* (1957).

Case 7, the mother of Cases 5 and 6, was the only adult in this series, and probably acquired her infection from close contact with her children. Case 10 again showed only abdominal symptoms with no obvious respiratory signs: *Sh. sonnei* was not isolated from the stools.

The finding of *Sh. sonnei* and adenovirus type 7 together in a high proportion of stools was unexpected. Our investigation of 50 normal children's stools in a neighbouring town revealed only one adenovirus, type 6. Most of the outbreaks previously described have been in closed communities, such as a school (Kendall *et al.*, 1957; Munro-Ashman *et al.*, 1958) and Army recruit units (Hilleman *et al.*, 1955), though van der Veen and van der Ploeg (1958) described outbreaks of adenovirus types 3 and 4 in the general population of Waalwijk, in Holland. Our results substantiated van der Veen and van der Ploeg and other workers' findings of the suitability of faeces for the isolation of adenovirus. Virus isolations were possible, though the faecal samples were not frozen during transmission to the laboratory. The need for keeping HeLa-cell cultures for at least 28 days was justified by the length of time it took for specific degeneration to occur in most of our cases (see Table I).

Cases 1, 2, and 10 suggest that adenovirus type 7 by itself can cause diarrhoea and vomiting. The inter-relationship between *Sh. sonnei* and adenovirus type 7 in cases where they occurred together was difficult to interpret. It is noteworthy that all cases, except one adult contact, were aged 5 years or under. It is possible that in this community *Sh. sonnei* and adenovirus were circulating together, but only in the younger susceptible children did adenovirus establish itself and superimpose respiratory symptoms on the enteric condition. Alternatively, the adenovirus infection may have brought to light a latent carriage of *Sh. sonnei*. As the number of cases was small further speculation would be unwarranted, but these results indicate that cases of Sonne dysentery, especially among younger patients, need further investigation with a view to establishing how commonly adenoviruses are associated with *Sh. sonnei*.

The evidence showed that adenovirus type 7 and *Sh. sonnei* were associated in the Newbiggin and Ashington area. This type of association was not found in 20 cases of Sonne dysentery or 20 cases of non-bacterial diarrhoea from other areas, though the investigation was carried out at the same time of the year. The frequency of adenovirus isolation in our group

was very significantly higher than in a control group of normal children—one adenovirus type 6 isolated from 50 children.

The outbreak described could be one of dual aetiology, some cases of diarrhoea resulting from adenovirus infection, others from *Sh. sonnei*, while a third group were infected with both agents and displayed respiratory as well as alimentary symptoms.

Summary

An outbreak of diarrhoea and vomiting is described in which adenovirus type 7 was isolated from the stools in 10 cases. Six of them were associated with *Sh. sonnei* and a number of these showed respiratory symptoms.

All cases except one adult contact were aged 5 years or less.

Cases of Sonne dysentery and non-bacterial diarrhoea, in this age group but from other areas, were examined for the presence of adenovirus with negative results.

The faeces of 50 normal children were also negative, with one exception when an adenovirus type 6 was isolated.

The possible role of adenovirus in this outbreak is discussed.

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REFERENCES

- Hilleman, M. R., Werner, J. H., Adair, C. V., and Dreisbach, A. R. (1955). *Amer. J. Hyg.*, **61**, 163.
 Kendall, E. J. C., Riddle, R. W., Tuck, H. A., Rodan, K. S., Andrews, B. E., and McDonald, J. C. (1957). *Brit. med. J.*, **2**, 131.
 Kjellen, L., Sterner, G., and Svedmyr, A. (1957a). *Acta paediat. (Uppsala)*, **46**, 164.
 — Zetterberg, B., and Svedmyr, A. (1957b). *Ibid.*, **46**, 561.
 Munro-Ashman, D., Gardner, P. S., Taylor, C. E. D., and McDonald, J. C. (1958). *Lancet*, **2**, 121.
 Ramos-Alvarez, M., and Sabin, A. B. (1958). *J. Amer. med. Ass.*, **167**, 147.
 Sommerville, R. G. (1958). *Lancet*, **2**, 1347.
 van der Veen, J., and van der Ploeg, G. (1958). *Amer. J. Hyg.*, **68**, 95.
 White, G. B. B., Gardner, P. S., and Hope Simpson, R. E. (1957). *Brit. med. J.*, **1**, 381.

"It is very gratifying to report that the shoplifting research project, which was mentioned last year as having good prospects for the future, is now actually under way. This has been made possible by donations from the Onslow-Whiting Research Trust and the Charles Henry Foyle Trust, with the addition of contributions from Messrs. Marks and Spencer, the John Lewis Partnership, and others. More money is still needed before the research can be completed; embarking on it at all is an act of faith which it is hoped will prove to be justified. . . . The project will take the form of a detailed investigation into the social background and personalities of convicted shoplifters. The main lines of inquiry in the early stages of the work were: (1) establishing contact with security officers or managers of large stores; (2) analysing detailed questionnaires of 100 shoplifters seen by the Medical Officer at Holloway Prison; (3) making a bibliography of relevant literature. The next stage of the inquiry will be the collection of information from the court records of three different areas to be covered in the survey, and from relevant documents at Scotland Yard." (*Annual Report, July 1958-June 1959*, Institute for the Study and Treatment of Delinquency.)