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EPIDEMIOLOGICAL AND VIROLOGICAL STUDIES ON OUTBREAKS OF ACUTE GASTROENTERITIS ASSOCIATED WITH ROTAVIRUS IN PRIMARY SCHOOLS IN OSAKA

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SUMMARY There have been three recent outbreaks of acute gastroenteritis in primary schools in Osaka prefecture caused by rotavirus: one in a school (TA) in April, 1974 and two in two other schools (TE and K) in May 1975.

The morbidity from the disease was 2.4–15.8% for all age groups in the schools, and 20.1–34.1% for a certain age group. The disease lasted for 4 (K) to 14 days (TE). The first cases in schools TA and TE were followed by successive cases. The main clinical symptoms were higher frequencies of diarrhea (68–71.4%) and fever (78%) than in winter vomiting disease. There was no difference in the incidences of the disease in boys and girls. From observation on the disease in individual families, the incubation period seemed to be 3 days. Using paired sera and Neonatal Calf Diarrhea Virus (NCDV) as antigen, positive seroconversion was demonstrated by the complement fixation test and marked seroconversion by the indirect fluorescent antibody technique. With these techniques, specific antibody to NCDV was detectable in the sera from an early stage of illness. Rotavirus was found on electron microscopic examination of some fecal specimens of patients in TA, but not in those of patients in TE or K, although adenovirus was isolated from one patient. A serological survey of healthy children aged 0 to 12 showed that rotavirus is a common virus in Osaka.

INTRODUCTION

Rotavirus, which has been termed reovirus-like agent, duovirus or infantile gastroenteritis

virus (Flewett and Woode, 1978; McNulty, 1978) was established as the etiological agent of infantile acute gastroenteritis with diarrhea (Bishop et al., 1974; Flewett et al., 1974a; Kapikian et al., 1976; Konno et al., 1977) as well as of diarrheal disease in adults (von

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Bonsdorff et al., 1976, 1978). Furthermore, outbreaks of acute gastroenteritis associated with rotavirus have been reported among the children in public facilities such as a nursery school (Kitawaki and Funahashi, 1978) and primary schools (Hara et al., 1976; 1978).

Recently, we performed virological and epidemiological investigations on epidemic gastroenteritis, including winter vomiting disease, which resembled food poisoning, but showed no detectable specific etiological bacteria. Respiratory illness with enteritis among school-age children was also studied, because it was thought likely that most cases were associated with infection by viruses, such as rotavirus, parvovirus-like agents or other viruses (Ramos-Alvarez and Olarte, 1964; Yow et al., 1970; Schreiber et al., 1977; Steinhoff, 1978).

Since there are few detailed reports of outbreaks of rotavirus infection associated with acute gastroenteritis in school-age children, this paper reports outbreaks of this disease caused by rotavirus in three primary schools and also a seroepidemiological survey showing the prevalence of rotavirus in Osaka.

MATERIALS AND METHODS

1. *Virus*

Neonatal calf diarrhea virus (NCDV), Lincoln strain, was kindly supplied by Dr. M. Kodama, National Institute of Animal Health, Tokyo, and was grown in primary bovine embryonic kidney (BEK) cells. Cells infected with the virus were harvested when cytopathic effects (CPE) became maximal, and centrifuged at low speed after three cycles of freezing and thawing. The supernatant fluid was treated with an equal volume of fluorocarbon (Daifron S3, Daikin KK, Japan), and centrifuged at low speed. The specimen was layered on a small volume of 45% sucrose solution and centrifuged at 35,000 rpm for 90 min. The precipitate was resuspended in phosphate buffered saline (0.01 M, pH 7.0) at one fiftieth of its original volume, and stored at -70°C until use.

2. *Cells*

Primary BEK cells were grown in Eagle's minimal

essential medium (MEM) containing 3% newborn calf serum and maintained in MEM without serum after virus inoculation. HEP-2, FL and green monkey kidney cells were grown in MEM containing 7% calf serum and maintained in the same medium with 2% calf serum after inoculation of specimens from patients.

3. *Virus isolation*

Throat washing and 10% emulsions of fecal specimens with Hanks' balanced salt solution were centrifuged at 3,000 rpm and 8,000 rpm, respectively, for 30 min at 4°C . The supernatants were mixed with antibiotics and inoculated into cell cultures. CPE were used as a marker of virus growth.

4. *Serological tests*

The complement fixation (CF) test was carried out for detection of antibody to NCDV in sera of patients and healthy children, using 4 units of antigen. Sera were inactivated by heating at 56°C for 40 min before the test. The indirect fluorescent antibody (IFA) technique was carried out by the method of Blacklow et al. (1976).

5. *Electron microscopic (EM) observation*

Fecal extracts were treated with fluorocarbon as mentioned above and then small samples were layered on 3 ml of 45% sucrose solution in a centrifuge tube and centrifuged at 30,000 rpm for 150 min in an SW 50.1 rotor of a Spinco model L ultracentrifuge.

The resulting pellet was suspended in two or three drops of distilled water, applied to a carbon coated-formvar-400 mesh grid with wire loop and examined in a Hitachi model HU-11B electron microscope after negative staining with 2% phosphotungstic acid (PTA) at pH 7.0. Rotavirus from a case with infantile gastroenteritis was prepared similarly and used as a control.

6. *Serum specimens from healthy children*

Sera from healthy children of various ages were collected in 1972 and 1975-76, and examined for CF antibody to NCDV. Most specimens were kindly provided by Dr. K. Kawakami, Department of Pediatrics, Osaka Medical College, Osaka.

7. *Clinical symptoms*

Clinical symptoms were surveyed by question-

naires to all patients. The symptoms in two schools (TE and K) were compared with those in schools YO and TS during outbreaks of winter vomiting disease in the winter and spring, respectively.

RESULTS

1. Outbreaks of acute gastroenteritis

Outbreaks of acute gastroenteritis which occurred among schoolchildren of three primary schools in Osaka, school TA in April, 1974, and schools TE and K in May, 1975 were found to have been caused by rotavirus, as shown in Table 1. All the cases occurred in warm spring weather. The numbers of cases, scored by data on the onset of the disease, are shown in Fig. 1a, b and c, for TA, K and TE, respectively. The morbidity in TA was 6.9% (63/936), but that of children of 11-12

years old was 29% (31/107). Two peaks of cases were seen, suggesting successive outbreaks of the disease. In school TE (Fig. 1c) the morbidity of all the children was 15.8% (249/1,571), while that of children of 9-10 years old was 20.1% (49/244). In this school the first outbreak was large and there were second and third outbreaks, and whole lasting for two weeks. On the other hand, in school K (Fig. 1b), cases were restricted to one class with a morbidity of 34.1% (14/41), and the outbreak was over within 4 days.

The morbidity was not related to the sex of the cases. One teacher in school TE and the families of some of the patients also developed similar acute gastroenteritis.

2. Clinical symptoms

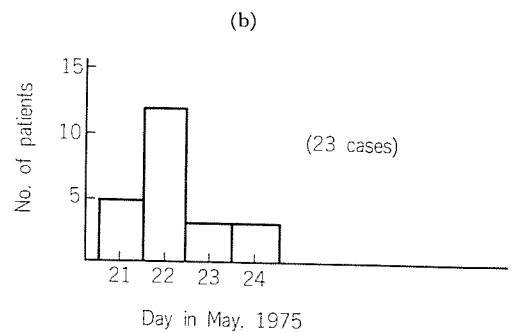
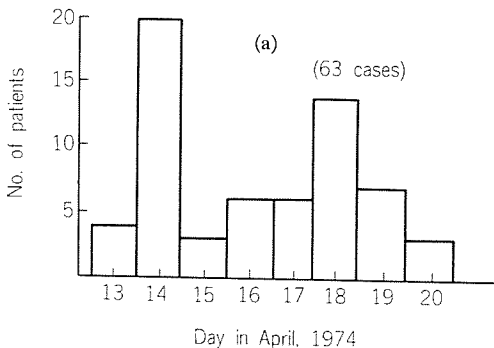
The clinical symptoms observed in the out-

TABLE 1. Outbreaks of acute gastroenteritis associated with rotavirus in Osaka

Primary school	Outbreak	No. of patients	Morbidity	
			School	Age group
TA	April, 13-20 1974	63	6.9% (63/936) ^a	11-12 years 29.0% (31/107)
TE	May, 14-31 1975	249	15.8% (249/1,571)	9-10 years 20.1% (49/244)
K	May, 21-24 1975	23	2.4% (23/950)	6-7 years ^b 34.1% (14/41)

^a Numbers in parentheses indicate No. of patients / No. of students.

^b in one class.



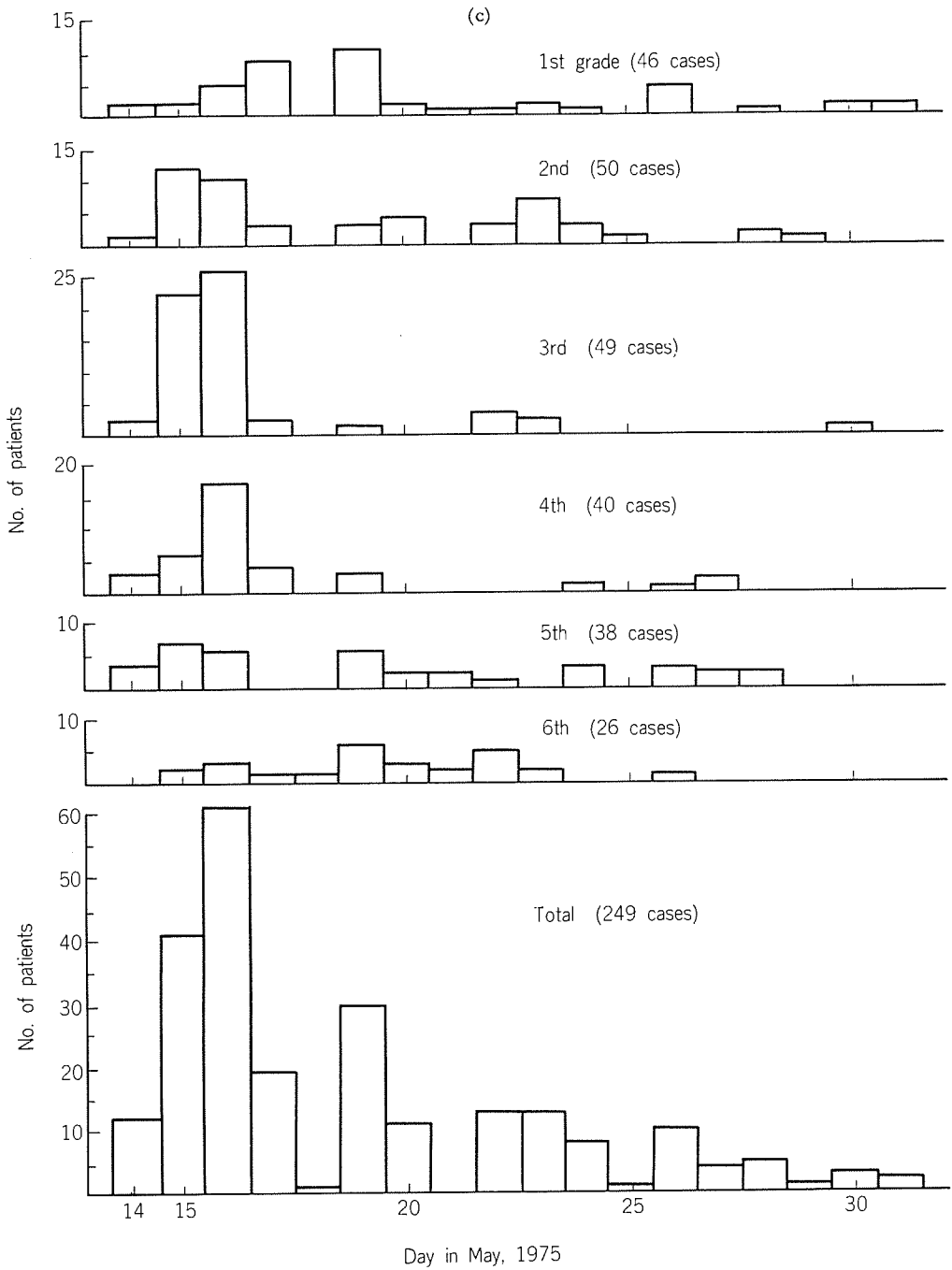


FIGURE 1. Outbreaks of acute gastroenteritis associated with rotavirus in primary schools.
a: TA school, b: K school, c: TE school.

breaks in schools TA and K are summarized in Table 2. In schools TS and YO the frequencies of patients complaining of abdominal pain, diarrhea and fever were lower than those in schools TA and K, where the etiological agent was determined to be rotavirus. Usually the duration of diarrhea was 1 or 2 days, the maximum duration being 4 to 6 days.

3. Virus isolation

For virus isolation, 25 throat washing and 30 fecal specimens were taken from cases in the schools, but only one strain of adenovirus was isolated from a fecal specimen of a patient in school TA. No bacteria that could be suspected of causing the diseases were isolated.

4. Electron microscopic examination

Fecal specimens of affected children in school TA were examined by EM. Rotavirus particles were detected in one of the five pooled fecal extracts as shown in Fig. 2a. Virus particles of about 70 nm diameter were observed with intact and empty particles. These particles were morphologically quite similar to rotavirus particles obtained from a case of infantile gastroenteritis, as shown in Fig. 2b.

No other virus particles could be observed by EM. Because of the shortage of specimens, EM observations were not performed on samples from schools TE and K.

5. Serological examination

Since NCDV is antigenically related to human rotavirus (Flewett et al., 1974b, Thouless et al., 1977), it was used as antigen in CF tests on paired sera. The results are shown in Fig. 3. With a few exceptions, a rise in specific antibody was observed between the acute and convalescent phases. Even at an early stage of the disease, most cases already had CF antibodies. The levels of antibodies in the two schools were much increased, when they were measured around 15th day after the onset of the disease. The level of antibodies in the other one did not show a distinct increase in CF test, when measured after around 25th day, but a distinct rise in IFA test, as shown in Table 3 was observed.

6. Distribution of antibodies against rotavirus in sera from healthy children

To investigate the prevalence of rotavirus in Osaka, we measured the levels of antibody to

TABLE 2. *Clinical symptoms of epidemic acute gastroenteritis in Osaka*

Primary school	TA	K	TS	YO
Occurrence	April 1974	May 1975	May 1976	Nov. 1976
Diagnosis	Viral gastroenteritis	Viral gastroenteritis	Like winter vomiting disease	Winter vomiting disease
Etiological agent	Rotavirus	Rotavirus	Unidentified	Unidentified
No. of patients	63	23	25	32
Clinical symptoms				
Vomiting (%) ^a	82.0	28.6	65.4	84.4
Abdominal pain (%)	77.7	— ^b	30.8	34.4
Diarrhea (%)	68.0	71.4	42.3	15.6
Nausea (%)	64.0	42.9	61.5	53.1
Anorexia (%)	—	—	65.4	78.1
Fever (%)	78.0	—	53.8	53.1

^a No. of patients with symptoms / Total no. of patients (×100).

^b Not investigated.

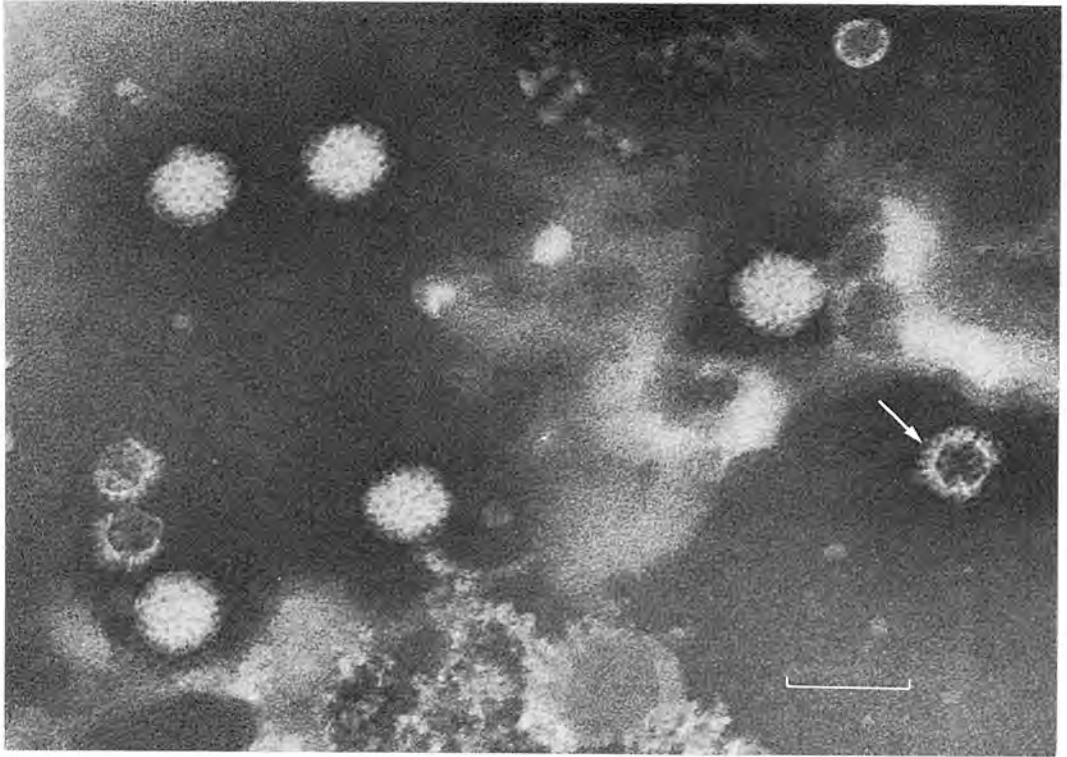


FIGURE 2. Rotavirus particles in fecal extracts. a: Fecal specimens from patients with acute gastroenteritis in TA school. b: from a case of infantile gastroenteritis. The arrow shows an empty virion. The bar represents 100 nm.

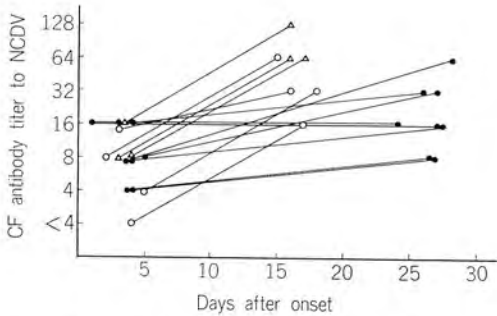
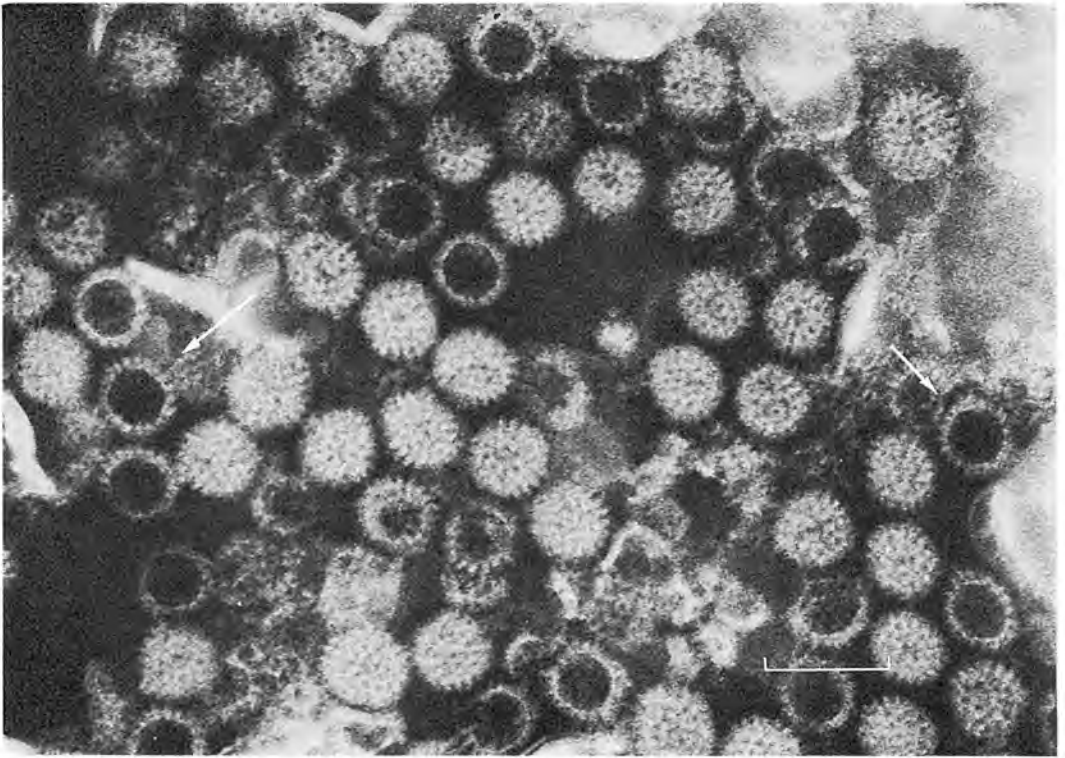


FIGURE 3. Antibody response to NCDV in paired sera from patients with acute gastroenteritis. Serum specimens were collected from TA (●—●), K (△—△) and TE (○—○) school.

TABLE 3. Comparison of antibody titers to NCDV tested by CF and IFA

Patient	School	Day after onset ^a	NCDV antibody titer	
			CF	IFA
IK	TA	1	16	128
		24	16	512
KY	"	4	8	32
		27	16	512
KM	"	5	8	16
		28	64	512
OM	"	3	16	32
		26	32	512
YY	"	4	8	128
		27	32	512
AY	TS	2	<4	<4
		21	<4	<4

^a Interval between onset of disease and preparation of sera.



b

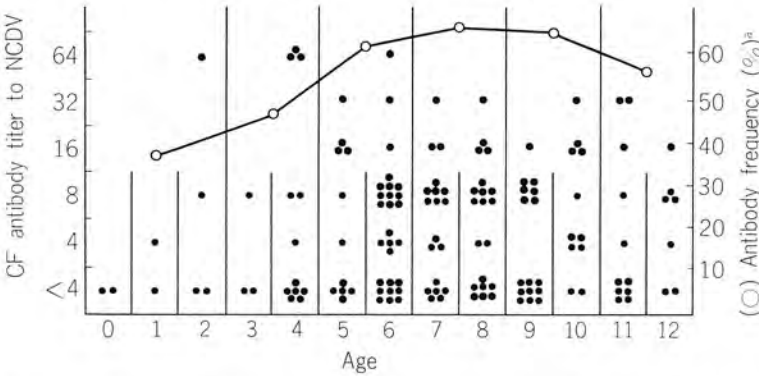


FIGURE 4. Distribution of antibody to NCDV in the sera from healthy children collected in 1975-76. a: No. with positive (1:4 screening) antibody/No. tested ($\times 100$).

NCDV in sera from healthy children collected in 1972 and 1975-76. As shown in Fig. 4, antibody to the virus was found in 40-60% of

specimens from children of 6 to 12 years old, i.e., primary-school children, and in 35-40% of the other specimens. Findings were similar in sera collected in 1972, when no outbreaks of gastroenteritis associated with rotavirus in school-children had been reported.

DISCUSSION

Virological examinations have shown that rotavirus is an etiological agent of acute nonbacterial gastroenteritis not only in infants but also in school-children.

In the outbreaks of acute gastroenteritis

caused by rotavirus described in this paper, most of the cases were restricted to a certain age group or a certain class in school. This suggests that relatively close contact between patients and healthy children was necessary for infection. The duration of the outbreak in school K was only 4 days, but the durations in schools TA and TE, where there were second or third outbreaks, were one and two weeks, respectively. The mode of transmission of rotavirus among schoolchildren is not definitely known, but, since some of the patients developed upper respiratory symptoms, the respiratory route, as well as the oral route of transmission seems possible.

As in other viral infection, no difference in the incidences in boys and girls was observed. The occurrence of the same diarrheal disease in a teacher and the families of some patients indicates that rotavirus can also cause the same disease in adults. As shown in Table 2, the clinical symptoms of rotavirus infection in schoolchildren and adults were characterized by higher frequencies of diarrhea and fever than in winter vomiting disease. The seasonal difference between rotavirus infection in school children and in infants raises the question of the identity of the causative agents of these two types of gastroenteritis.

This question is difficult to solve, because so few virus particles were obtained from clinical specimens and because the virus cannot be cultivated *in vitro*. In 1978, Hara et al. reported an antigenic difference between the two agents.

For serodiagnosis of the disease with NCDV

as antigen, the IFA test (Table 3) was more sensitive than the CF test (Fig. 3).

This finding is consistent with the report of Blacklow et al. (1976). The presence of CF antibody against NCDV in most of the sera obtained at an early stage of illness indicates that the patients had previously been infected with rotavirus possessing common antigen with NCDV. Although the possibility of reinfection with rotavirus has been suggested by some investigators (Gomez-Barreto et al., 1976; Ørstavik et al., 1976), immunoglobulin classification of anti-rotavirus antibody in patients' sera is required.

A seroepidemiological survey on healthy children showed that rotavirus is a common virus in Osaka.

The etiological agents of the three outbreaks of acute gastroenteritis of schoolchildren were showed to be rotavirus, but studies are still required on several other outbreaks of similar diseases in which rotavirus was not demonstrated as the causative agent. For example, we have detected coxsackie virus type B-3 infection among schoolchildren with diarrhea (Oishi et al., 1978), and there may be yet other causative agents.

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