Aetiological and Clinical Studies on Adenovirus Infections in Nagasaki, Japan 1964-1966

Koichi FUTATSUKI

Department of Virology, Institute for Tropical Medicine, Nagasaki University,
(Director : Prof. Dr. Hideo FUKUMI)

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Abstract

During a period from June 1964 to February 1966 in Nagasaki, 676 patients with acute respiratory illness, diarrhea or skin rash were examined at the time of the initial visits, and in addition 145 of the cases were examined in approximately 2 to 4 weeks after onset of the illness. HeLa cells alone were employed throughout the study, and the total incubation period in each of the negative specimens was at least a total of 80 days through three passages, cultures being maintained in Hanks's B.S.S. containing 0.5% lact-albumin hydrolysate, 0.1% yeast extract, and usually 3 to 4% bovine serum. Forty-nine adenovirus strains were isolated from 48 of the cases examined. Types 2, 3 and 5 were the commonest, and types 1, 6 and 11 were also recovered. Type 3 was mainly obtained from school children in a sharp outbreak in the summer of 1965. All the strains of type 3 caused upper respiratory disease and the recovery of this type was always accompanied by a rise in both CF and neutralizing antibody. Types 2 and 5 occurred chiefly in infants in both the summers of 1964 and 1965 and were associated with 3 cases of diarrhea and 6 cases of exanthema in addition to upper respiratory illness. There was a neutralizing antibody rise in 40% of each of the groups excreting type 2 and 5 agent respectively. All strains of types 1,6 and 11, excepting one type 1 strain, were found in pre-school children in the winter of 1965-66, and in 3 cases of diarrhea besides upper respiratory disease. The 49 strains were obtained from 5.1% of 710 throat specimens collected, from 6.7% of 461 anal specimens, and from none of 117 eye specimens. A study of a limited number of the patients from whom both throat and anal specimen were collected indicates that adenoviruses were isolated with equal ease from throat and anal specimens. Those collected specimens were kept at 4°C or at room temperature in six hospitals and five practitioner's offices from the time of collection before they were transported to the laboratory only once or twice a week without ice. Some of them spent more than 5 days at 4°C or at room temperature before transported. There was little influence of specimens spending longer than 6 hours at 4°C or at room temperature not only on the isolation

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rate but on the time required for the first detection of the CPE. The optimal time for taking throat specimens for adenovirus isolation was the 4th day of illness, and that for taking anal specimens was the 4th day up to about 21st day. Serologically 18.4% of 136 patients were diagnosed as positive by the CFT. Although no adenovirus was isolated from any specimens of 206 adults, 13.5% of 37 adults were confirmed as adenovirus infection only by a rise in CF antibody titer. In addition to the adenovirus strains, enteroviruses were isolated from 29 patients.

Introduction

Since adenovirus was isolated for the first time by Rowe *et al.* (1953)[51] and Hilleman and Werner (1954)[29], the etiological role of adenoviruses in acute respiratory diseases has been studied by many investigators in the United States and other countries. In Japan, since Fukumi *et al.* (1957)[14] reported four cases of pharyngoconjunctival fever due to adenovirus, many investigations of various respiratory illnesses due to adenovirus have been carried out (Matumoto, 1963)[42]. It is now very clear that adenovirus is a causative agent of pharyngoconjunctival fever, which has been recognized to be accompanied by gastro-intestinal symptoms for some time (Fukumi *et al.*, 1958[16]; Duncan and Hutchinson, 1961[7]; Kaji *et al.*, 1961[37]). Some investigators (Hirayama, 1959[30][31]; Kamiya *et at.*, 1961[39]; Takatsu, 1961[54]) have reported that adenoviruses were isolated from infants with diarrhea of white faeces, and the etiological relation of the adenoviruses to infantile diarrhea, especially "pseudocholera infantum"* is nowadays drawing attentions. On the other hand, recovery of a cytopathogenic agent, which was later classified as adenovirus (Bell *et al.*, 1956[1]), from faeces of an infant with rash resembling that of roseola infantum by Neva and Enders (1954)[46] gave rise to a suspicion about the relation between adenovirus and exanthematous illness, and nowadays the relationship has become one of the most important problems in the adenovirus infections. Recently Bell (1965)[3] has found that 5 per cent of the cases of aseptic meningitis and 10 per cent of those of encephalitis are associated only with an adenovirus infection, principally type 2, and has suggested that the adenoviruses are implicated in infections of the central nervous system. Thus, many researches have been made, but the role of the adenoviruses in the etiology of acute respiratory illnesses, diarrhea in children, and exanthema etc. is not yet fully understood, and in addition some part of ecological knowledge of the adenovirus infection still remains to be studied. To make matters worse, in Kyushu area, Japan, only a few of such detailed studies have been carried out, and in Nagasaki any study has never been done.

The present report describes the findings in the studies carried out with a view to putting anything in requisition of prevention of an illness or an epidemic, by investigating (1) the relation between the types of adenovirus and the clinical symptoms, (2) antibody responses to adenovirus infections according to the day of the illness, and (3) the excretion period of the adenoviruses in various routes of the patients. There was clearly the need for

* The disease will be explained in Discussion.

collecting specimens as many as possible on different days after onset of the illness from the patients of various age groups suffering from acute respiratory illnesses, diarrhea or skin rash etc.. However, no effort was made to examine a control group of the general population. Between June, 1964, and February, 1966, in Nagasaki 1,288 specimens for virus isolation and 136 paired sera for serological tests from 676 patients with so-called "cold" syndrome mainly in summer season and with diarrhea mainly in winter season were examined for adenovirus. No particular attempt was made to examine other viruses. To add to the results obtained in the subjects, this paper reports the findings in the problems of the delay in delivery of specimens to the laboratory and the optimal time for taking the specimens, and also describes the detailed clinical features of 6 cases with exanthema.

Materials and Methods

Condition of Patients

Specimens for virus isolation were obtained from 676 infants, children and adults in Nagasaki during the period from June 11, 1964, up to February 20, 1966. Of these, 403 infants and children were seen in the outpatient clinics of six hospitals* and five practitioners** mainly for acute respiratory illness or diarrhea, and 190 adults were seen in the same outpatient clinics for acute respiratory illness. Only 30 children and 16 adults were hospitalized in 5 of the 6 hospitals and in 2 of the 5 practitioners' wards for lower respiratory tract infection, and 37 infants were admitted to the ward of an asylum*** for acute respiratory disease or exanthema. Paired serums for serological studies were obtained from 136 of the 676 individuals from whom the specimens for virus isolation were collected.

Clinical Observations and Cases

For the studies, detailed clinical observations and interrogations of the outpatients were conducted at the time of the initial and the second visits, and those of the inpatients were conducted everyday. These were made by physicians of each hospital and each clinic. All patients were classified into the following disease categories.

(**A**) *Upper respiratory tract infection* (U.R.T.I.)

This is classified in 5 syndromes according to the classifications described dy Dowling and Lefkowitz (1963)[6] and Parrott *et al.* (1963)[47]:

(**a**) *coryzal syndrome*, which is characterized by increased nasal discharge and occulusion and rarely accompanied with fever;

(**b**) *pharyngeal syndrome*, in which the prominent symptoms are sore throat, redness of the pharynx and swelling of the tonsils, often with fever, cough, coryza and mild systemic symptoms;

(**c**) *pharyngoconiunctival fever* (P.C.F.) *syndrome*, in which the pharyngeal syndrome is present in addition to conjunctivitis;

(**d**) *influenza syndrome*, which has as its outstanding feature the constitutional symptoms such as chilliness, headache, general aching, malaise and anorexia in a high percen-

* Mitsubishi Heavy Industries Ltd. Nagasaki Shipyard and Engineworks Hospital, Nagasaki Atomic Bomb Hospital, Nagasaki Municipal Citizen Hospital, Nagasaki Municipal Nagasaki Hospital, Nagasaki University Hospital, and Saisei-Kai Hospital.

** Inoue Hospital, Satomi Pediatric Clinic, Takamori Clinic, Sumiyoshi-Chuo Hospital, and Zeshinkai Takahara Hospital.

*** Nagasaki Municipal Suckling Asylum

tage of coryzal or pharyngeal syndrome;

(**e**) *herpangina syndrome*, wich is characterized by vesicles in the pharynx and on the hard and soft palate.

(**B**) *Lower respiratory tract infection* (L.R.T.I.) This is also classified in 3 syndromes:

(**f**) *pleurodynial syndrome*, which is characterized by the sudden onset of severe pain in the thorax aggravated by breathing;

(**g**) *bronchitis*, which includes croup;

(**h**) *pneumonia*, which is confirmed radiologically.

(**C**) *Fever of unknown origin* (F.U.O.)

This is characterized by high fever alone, showing no symptom of acute respiratory disease, aseptic meningitis, encephalitis, or paralysis.

(**D**) *Exanthema*

This includes all exanthematous infections resembling rubella, measles, scarlet fever, exanthema subitum, urticaria, or roseola infantum, with or without fever, pharyngitis, or conjunctivitis.

(**E**) *Diarrhea*

This includes diarrhea associated with the coryzal or the pharyngeal syndrome, but when diarrhea is not so severe and the coryzal or the pharyngeal syndrome is prominent the patients are not included in this diarrhea group. *Pseudocholera infantum* is also included, which will be well explained in Discussion.

(**F**) *Other diseases*

This includes conjunctivitis, meningitis, myelitis, and the cases from whom no answer of clinical observation was received.

Collection of Specimens

All of serum, throat, anal and conjunctival materials were tried to be taken from each patient during the acute phase of illness, but throat swabs or pharyngeal garglings were mainly collected from the patients with acute respiratory illness and anal swabs or faeces from the cases with diarrhea. Moreover approximately 2 to 4 weeks after onset of the illness, efforts were made to obtain the second those samples only to fail in almost four-fifths of the cases, owing to the failure of the patients to return at the designated time. At the time of the initial visits of patients, though some specimens were taken more than 7 days after onset of the illness, 584 throat, 364 anal, 94 eye and 370 serum specimens were collected from 676 patients. The number of the specimens on the second visits were 126 throat, 97 anal, 23 eye and 136 serum specimens, which were collected from 145 among the 676 patients. Thus, 1,288 materials for virus isolation and 136 paired serums for serological tests were obtained.

Throat, anal and conjunctival swab specimens were collected by use of the cotton swabs which were placed in the test tubes with 3 ml of bonito infusion broth containing 1,000 units of penicillin and 1,000 μg of streptomycin per ml, and throat washings were obtained by gargling with 10 ml of the same transport medium. Faeces were put into the tubes with 10 ml of bonito infusion broth containing 25% glycerin, 1,000 units of penicillin and 1,000 μg of streptomycin per ml. These specimens obtained in the hospitals and clinics were kept at 4°C or sometimes at room temperature from the time of collection before they were transported to the laboratory only once or twice a week without ice by road. Some of them spent more than 5 days at 4°C and several days at room temperature before transported. In the laboratory they were frozen at -20°C and stored until tested. Approximately 60% of specimens were inoculated into tissue cultures within 2 weeks after collection, but the others were

stored frozen at -20°C for several months until inoculated.

Specimens of blood were taken in the test tubes and kept at 4°C, until they were transported to the laboratory and separated from the clot. The serums were kept at -20°C until tested.

Tissue Culture

Throughout the study, a strain of HeLa cells was employed. HeLa cells were kindly supplied by Dr. M. Shingu, Department of Microbiology, Kurume University School of Medicine, Fukuoka, Japan. During the whole of the work, the cultures were grown at 36.5°C in Hanks's Balanced Salt Solution (B.S.S.) containing 0.5% lactalbumin hydrolysate and 10% bovine serum. Test tube cultures for virus isolation were prepared by inoculating to culture tubes with 0.6 to 0.8 ml of a HeLa cell suspension containing approximately 200,000 cells per ml. The cultures ready for use, which were usually 3 days old, were washed once with 2 ml of Pack's B.S.S. and the washed cell sheets were then overlaid with 1.0 ml of Hanks's B.S.S. containing 0.5% lactalbumin hydrolysate, 0.1% yeast extract and 3 to 4 per cent bovine or calf serum. For virus titration and neutralization test, test tube cultures were prepared by inoculating with 0.6 ml of growth medium containing 400,000 cells per ml. After 24 to 36 hours of incubation the tube cultures were washed twice with 2.0 ml of Pack's B.S.S., and 0.8 ml of Hanks's B.S.S. containing 0.5% lactalbumin hydrolysate and 0.1% yeast extract (Y.L.H.) was added to each tube, and then the tubes were used. All mediums contained 200 units of penicillin and 200 μg of streptomycin per ml.

Virus Isolation Procedures

Faeces in transport medium were homogenized with Hanks's B.S.S. to give a 10 to 20 per cent emulsion. They were then centrifuged at 3,000 r.p.m. for 30 minutes 3 times. To the supernatant fluid obtained from the third centrifugation, antibiotic solution was added to give the final concentration per ml of 1,000 units of penicillin, 1,000 μg of streptomycin and 400 units of mycostatin, and then the samples were held at 4°C overnight. The specimens were again centrifuged at 3,000 r.p.m. for 30 minutes, and 0.1 ml of the supernate was inoculated into 3-day-old tube cultures on maintenance medium which were prepaired. Two HeLa tube cultures were used for one specimen. The nutrient fluid was changed after incubation for 2 hours at 35°C.

Throat, anal and conjunctival swabs and pharyngeal washings in bonito infusion broth were centrifuged at 3,000 r.p.m. for 30 minutes, the supernate was removed, and antibiotic solution was added in the final concentration of 1,000 units of penicillin, 1,000 μg of streptomycin and 400 units of mycostatin per ml. The samples were kept at 4°C for 4 hours to overnight, and then centrifuged at 3,000 r.p.m. for 15 minutes, and 0.2 ml of the supernate was inoculated into each of 2 HeLa tube cultures of 3-day-old on maintenance medium.

All tubes were incubated at 35°C in a stationary position for at least 28 days, and examined microscopically 3 to 4 times a week for cytopathic effect, changing the medium every 4 to 5 days. If any cytopathogenicity was observed, at the height of cellular degeneration the fluid and cells were harvested by freezing and thawing 6 times for next passage and storage. If no cytopathogenicity was noted for minimum of 28 days, two additional passages were carried out by freezing and thawing the cultures 6 times and by inoculating 0.2ml of the fluid into fresh monolayers

to extend the incubation period to at least 80 days. The tubes that proved negative after 25 days of incubation in the third passage were discarded. When cytopathogenic changes in the HeLa cells were not suggestive of adenovirus but rather of enterovirus occurred, the cultures were harvested by freezing and thawing once and identified with polio-antiserums. In all critical and positive specimens the isolation procedure was repeated from the original materials.

Identification of Adenoviruses

Neutralization tests were performed exclusively in 2-day-old HeLa cell cultures for typing any isolates. The technique for adenovirus was a modification of those described by Rowe *et al.* (1955)[52], Grayston *et al.* (1956)[20], Rafajko (1964)[48], Rose (1964)[49], Wigand *et al.*(1965)[57] and Ellis *et al.* (1966)[8].

The viruses isolated were propagated in HeLa cell cultures which were incubated for a few days after the cells had shown a 4 + cytopathic changes. The infected cells and maintenance medium were harvested, frozen and thawed 6 times, and the cell debris were removed by centrifugation at 3,000 r.p.m. for 15 minutes. Serial twofold dilutions of the isolates were prepared in Hanks's B.S.S., and 0.2 ml of each dilution was inoculated into 2 cultures containing 0.8 ml of the serum free maintenance solution. The cultures were examined for CPE 48 hours after inoculation, and the endpoint was defined as the highest dilution of virus producing a 4+ CPE. Some isolates produced very little cellular change and were allowed additional time for incubation. In these cases, the tubes were read for CPE on the 4th or the 5th day, or later.

The unknown viral agent was diluted in Hanks's B.S.S. to contain twice the virus necessary to produce the endpoint CPE. 0.25 ml of the diluted virus was mixed in individual tubes with the same amount of each type-specific horse antiserum against adenovirus types 1 to 18, diluted 1:10. A virus control tube was prepared by mixing 0.3 ml of the diluted virus with equal volume of Hanks's B.S.S., and a 10^{-1} virus control was also prepared by mixing 0.1 ml of the first virus control suspension with 0.9 ml of Hanks's B.S.S.. After the serum-virus mixtures and the controls were held at 35°C for 2 hours and then at room temperature for 2 to 3 hours, 0.2 ml of each of the mixtures and the controls was inoculated into 2 tube cultures containing 0.8 ml of the serum free maintenance medium. The cultures were incubated at 35°C in a stationary position for 4 to 5 days and inspected for CPE. Neutralization was considered as positive when antiserum-containing tubes showed a complete prevention of cytopathogenic change 48 hours after the virus control had reached a 4+ reading.

Identification of Polioviruses

The method was carried out according to a modification of the techniques described by Kono *et al.* (1958)[41], Melnick *et al.* (1964)[43], Schmidt (1964)[53], and Ellis *et al.* (1966)[9]. Specific typing antisera against 3 types of poliovirus were also kindly given by Dr. M. Shingu. They were sera from monkeys immunized with type 1 (Mahoney), type 2 (Sabin 2) and type 3 (Sabin 3), and from rabbits immunized with type 1 (Mahoney), type 2 (MEF 1) and type 3 (Saukett). All these sera except Anti-Mahoney monkey serum had neutralizing antibody titers of over 1:1,000 against 100 TCD_{50} of homologous virus. 0.25 ml of each of antisera and pooled one of types 1 to 3 containing 20 antibody units per 0.1 ml was mixed with the same amount of tissue

culture fluid diluted to contain 1,000 TCD_{50} per ml 4 days after inoculation. A virus control was also prepared. The mixtures and the virus control were incubated at 35° C for 90 minutes, and 0.2 ml of these was inoculated into each of 2 tubes of 2-day-old cultures. This 1,000 TCD_{50} challenge virus was diluted 1:10, 1:100, 1:1,000, and 1:10,000 for the virus titration, and 0.1 ml of each dilution was inoculated into 4 tubes. Results were read on the 4th day, and complete prevention of CPE was interpreted as a positive neutralization.

Complement Fixation Procedure

The tests were simultaneously carried out with acute and convalescent sera of the same patient on plastic haemagglutination plates, according to the same method as that described by the National Institute of Health, Tokyo[58]. Isolated adenovirus types 3 and 5 were employed for complement-fixing antigen, which was prepared by propagating the viruses in bottle cultures of HeLa cells. The virus inoculum had been adjusted so that approximately 5 to 7 days were required for complete cytopathogenic changes. The bottles were held for 2 to 3 days after all the cells had become detached, the cells and medium were frozen and thawed 6 times, centrifuged, and the supernatant fluid was harvested. The antigen was made by pooling equal amounts of type 3 and 5 adenovirus supernatant fluid, which had been inactivated by heating to 56°C for 30 minutes. Serum was also inactivated at 56°C for 30 minutes, and twofold dilution was made.

On the plastic plates were dropped serum dilution in 0.02 ml (1 drop), 4 units of antigen in the same amount, and 2 full units of comlement in 0.04 ml (2 drops). After overnight incubation at 4°C the hemolytic system, which consisted of equal volumes of a 2.5% suspension of washed sheep cells and 20 units of sheep cell hemolysin, in 0.04ml (2 drops) was added. The plates were then reincubated at 37°C for 60 minutes, and the test was read after additional incubation at 4°C for 2 to 4 hours. The endpoints were read as reciprocal of the highest original dilution of serum producing a 3+ fixation. The diluent used throughout was Veronal-buffered saline solution. The initial serum dilution used was 1:4, except in some babies under one year. A four-fold or greater rise in antibody between the acute and the convalescent serum was interpreted to be a significant change.

Serum Neutralization Tests

Serum neutralization tests were carried out on paired sera against any virus isolated from that patient. Method for titrating neutralizing antibodies against adenoviruses was basically similar to that for typing adenoviruses. The sera were inactivated at 56°C for 30 minutes and twofold dilutions were made in Pack's B.S.S.. To 0.25 ml of each serum dilution was added 0.25 ml of virus dilution containing an amount of virus to produce 4+ cytopathogenic changes in 0.1 ml 48 hours after inoculation. The mixtures were allowed at 35° C for 2 hours and then 0.2 ml of each was inoculated into 2 tubes. A virus control and a 10^{-1} virus control were included. The tubes were incubated for 3 to 4 days and the test was read 24 hours after the virus control had shown a 4+reaction. The neutralizing titer of serum was expressed as reciprocal of the highest initial dilution showing greater than a 2+ difference between the cytopathogenic changes in the serum-containing tubes and the virus control. In case of slowly growing adenovirus, challenge virus was diluted to produce a 4+ CPE in 0.1 ml on the 4th day or

later.

Serum neutralization procedure for enteroviruses was also the same as that for typing polioviruses. 0.25 ml of each of twofold dilution of serum was mixed with the same amount of virus diluted to contain 100 TCD_{50} per 0.1 ml on the 4th day. The mixtures were incubated at 35°C for 90 minutes and 0.2 ml of each of the mixtures was inoculated into 2 tubes. The neutralizing titer was taken as reciprocal of the highest original dilution of serum showing a 1+ CPE 4 days after inoculation.

Results

Distribution of Disease Categories

The distribution of disease categories throughout the period of survey is shown in Table 1. During the summer season from June to October, 1964, 220 patients were included in the survey; 69% of the cases were in the U.R.T.I. group, 10% in the L.R.T.I. group, 3% in the exanthema group, 6% in the

Table 1. The distribution of disease categories, June, 1964, to February, 1966.

Month of onset of illness of patients	Disease categories						
	U.R.T.I.*	L.R.T.I.**	F.U.O.***	Exanthema	Diarrhea	Other Diseases****	Total
1964··· 6	1	1	—	—	—	—	2
7	—	3	—	—	—	—	3
8	71	3	3	3	2	1	83
9	50	9	7	4	11	8	89
10	29	6	—	—	—	8	43
11	—	—	—	—	—	—	—
12	1	—	—	—	—	1	2
1965··· 1	—	—	—	—	—	2	2
2	—	—	—	—	—	1	1
3	—	3	—	18	—	1	22
4	—	8	—	4	—	—	12
5	2	12	—	—	—	2	16
6	12	5	2	—	—	2	21
7	33	3	5	2	1	1	45
8	66	4	1	4	—	—	75
9	41	1	10	11	4	4	71
10	20	1	6	3	3	1	34
11	8	1	2	—	9	4	24
12	6	1	1	—	14	—	22
1966··· 1	4	1	2	—	67	1	75
2	13	2	1	—	16	2	34
Total	357	64	40	49	127	39	676

* Upper respiratory tract infection
** Lower respiratory tract infection
*** Fever of unknown origin
**** Meningitis, myelitis, conjunctivitis, etc.

diarrhea group, and 12% in the F.U.O. and the other diseases group. In the winter of 1964-65, the specimens were collected from only 5 patients, and besides in November, 1964, no case was examined. The number of the patients examined during the spring season from March to May, 1965, were 50; 46% of the cases were in the L.R.T.I. group, 44% in the exanthema group, and only 4% in the U.R.T.I. group. In the summer of 1965, 246 patients were examined; similarly in the summer of 1964, 70% of the cases were in the U.R.T.I. group; the other disease categories were responsible for 30% of the cases. 155 patients were examined in the winter of 1965-66; 68% of the cases were in the diarrhea group and only 20% in the U.R.T.I. group, while the patients with exanthema were not examined.

Virus Recovery

The number of specimens collected and the number of isolates during the period of the survey are summarized in the first part of Table 2, and the number of virus strains isolated and the number of patients having viruses in the latter part of Table 2. From 676 patients 710 throat, 461 anal and 117 eye specimens were collected. 36 adenoviruses and 17 enteroviruses were recovered from 710 throat swabs and garglings, 31 adenoviruses and 22 enteroviruses from 461 anal swabs and faeces, and none from 117 conjunctival swabs. These 67 adenoviruses and 39 enteroviruses were isolated from 73 patients. 48 of the 73 patients had adenoviruses; 25 were the patients from whom specimens were collected only once, and 23 were those from whom specimens were collected twice. Among these 23 patients, 3 had one or two adenoviruses and a poliovirus each on two different visits or from throat and anal specimens on the same day, 3 had a single adenovirus strain each not only during acute phase of illness but during convalescence, 12 had an adenovirus on the first visit only, and 5 had it on the second visit only. Among those 25 patients one had an adenovirus type 3 and an unidentified enterovirus, and the others

Table 2. Summary of virus recovery from 676 patients, June 11, 1964, to February 20, 1966.

		Tested No.	Adenovirus positive No.	Adenovirus							Enterovirus						Total
											Poliovirus			CB*4		E*5	
				I	II	III	V	VI	XI	?*1	I	II	III	III	VI	?*2	
Specimens	Throat	710	36	2	6	22	5	1	—	—	—	—	—	1	—	16	53
	Anal	461	31	1	5	15	6	1	1	2	1	1	2	—	1	17	53
	Eye	117	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	Total	1,288	67	3	11	37	11	2	1	2	1	1	2	1	1	33	106
Patients	Single virus infection		44	3	6	22	8	2	1	2	1	—	—	1	1	22	69
	Multiple virus infection*3		4	—	3	1	1	—	—	—	—	1	2	—	—	1	4
	Total	676	48	3	9	23	9	2	1	2	1	1	2	1	1	23	73

*1 ? = Adenoviruses assumed to be types of higher number than 18.
*2 ? = Viruses assumed to be coxsackieviruses or echoviruses.
*3 Two adenovirus strains (type 2 and 5) and a poliovirus type 3 were obtained from one patient, and an adenovirus and an enterovirus from each of 3 other patients.
*4 CB=Coxsackievirus B
*5 E = Echovius

Table 3. Laboratory findings among the patients from whom 2 or 3 viruses were isolated.

Case No.	Age	Clinical syndrome	Day of illness on which specimens taken	Virus isolated		Antibody response		
				Throat	Anal	Adeno-CF	Adeno-NT*	other-NT*
31	9 Mo.	Exanthema with pharyngitis	12	—	—	16	4	4
			23	+Adeno. 2	+Polio. 2	8	8	4
32	7 Mo.	PCF syndrome	10	+Adeno. 5	+Polio. 3	8	N. D.**	N. D.**
			21	+Adeno. 2	—	8		
34	5 Mo.	PCF syndrome	10	—	+Polio. 3	4	2	16
			21	+Adeno. 2	+Adeno. 2	8	2	64
472	6 Yr.	Pharyngeal syndrome	6	+Adeno. 3	+Entero. ?	N.D.***	N. D.***	N. D.***

* Against each isolated virus.
** Not done from want of an amount of serum.
*** Not done because of taking no serum in convalescence.

had an adenovirus strain each. Thus, four patients were excreting two or three different viruses; one had two strains of adenovirus type 2 and 5 with a poliovirus type 3, and the others had an adenovirus and an enterovirus each. The details of these cases, which could not be confirmed serologically in all of the cases, are given in Table 3. A total of 49 adenovirus strains were therefore recovered from the 48 patients. Of these strains, adenoviruses of types 2, 3 and 5 were 84%, and over half of these were type 3. The remainder of the adenovirus strains were types 1,6 and 11; in addition 2 strains were recovered, which have not yet been identified and are assumed to be adenoviruses of the higher numbered designations than 18.

39 isolates which were assumed to be enteroviruses were isolated from 29 patients. Four isolates which were obtained with adenoviruses from four patients were identified as polioviruses of type 1,2 or 3. Two isolates were identified as coxsackievirus type B3 and echovirus type 6, which were kindly performed by Dr. K. Ishii, National Institute of Health, Tokyo. The remainder could not be identified as polioviruses and are assumed to be coxsackieviruses or echoviruses, owing to their characteristics of CPE, resistance to temperature, and sensitivity to HeLa and monkey kidney cells and newborn mice. Thus, adenoviruses and enteroviruses were recovered from 73 of the 676 patients examined.

The Relation of Recovery of Adenoviruses to Season

Table 4 shows the distribution of the patients having viruses according to the month when the patients had their onsets of illness. It would be dangerous to discuss on the seasonal distribution of the viruses from such a small number of isolates and moreover from lack of isolations during the winter and the spring from October, 1964, to May, 1965, because of very few patients examined and most of them suffering from L.R.T.I. or exanthema, but some interesting points in this respect are observed from the study. Only 11 of 220 patients examined (5.0%) had adenoviruses in the summer of 1964 and 10 of 155 patients (6.5%)

Table 4. The frequency distribution of the patients having adenoviruses, according to the month of onset of the illness.

Month of onset of illness of patients	No. of patients tested	No. of patients having adeno-viruses	Adenovirus							Enterovirus Cox. Poliovirus			B Echo			Total No. of positive patients
			I	II	III	V	VI	XI	?	I	II	III	III	VI	?	
1964··· 6	2	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
7	3	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
8	83	9*	—	4	1	4	—	—	1	—	1	2	—	—	2	11**
9	89	2	—	—	—	2	—	—	—	—	—	—	—	—	2	4
10	43	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	2	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
1965... 1	2	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
2	1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
3	22	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
4	12	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
5	16	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
6	21	2	—	—	2	—	—	—	—	—	—	—	—	—	—	2
7	45	3	—	—	3	—	—	—	—	—	—	—	—	—	7	10
8	75	6	—	—	5	—	—	—	1	—	—	—	1	—	7	14
9	71	11	—	4	5	2	—	—	—	—	—	—	—	—	3	13**
10	34	5	1	—	4	—	—	—	—	—	—	—	—	—	—	5
11	24	2	1	—	—	—	1	—	—	1	—	—	—	1	1	5
12	22	3	—	—	2	1	—	—	—	—	—	—	—	—	—	3
1966... 1	75	3	1	1	—	—	—	1	—	—	—	—	—	—	1	4
2	34	2	—	—	1	—	1	—	—	—	—	—	—	—	—	2
Total	676	48*	3	9	23	9	2	1	2	1	1	2	1	1	23	73**

* Two adenovirus strains (type 2 and 5) with a poliovirus type 3 were obtained from one patient.

** One or two adenovirus strains and an enterovirus were obtained from each of 4 patients.

Table 5. Age distribution of the patients having adenoviruses.

Age	No. of patients tested	No. of patients having adeno-viruses	Adenovirus							Enterovirus Cox. Poliovirus			B Echo			Total No. of positive patients
			I	II	III	V	VI	XI	?	I	II	III	III	VI	?	
		%														
0-11 months	107	13*(12.1)	—	8	—	6	—	—	—	1	1	2	1	1	3	19**
1- 4 years	191	12 (6.3)	2	—	5	—	2	1	2	—	—	—	—	—	10	22
5-14 years	172	23 (13.4)	1	1	18	3	—	—	—	—	—	—	—	—	8	30**
15-81 years	206	0 (0.0)	—	—	—	—	—	—	—	—	—	—	—	—	2	2
Total	676	48*(7.1)	3	9	23	9	2	1	2	1	1	2	1	1	23	73**

* Two adenovirus strains (type 2 and 5) with a poliovirus type 3 were obtained from one patient.

** One or two adenovirus strains and an enterovirus were obtained from each of 4 patients.

had them in the winter of 1965-66, as compared with 27 of 246 patients (11.0%) having them in the summer of 1965. Adenoviruses of type 3 appeared most significantly in a sharp outbreak which took place during the summer season from June to October, 1965, but only one recovery of this type was made out of 220 patients examined in the summer of 1964. Adenoviruses of types 2 and 5 were predominantly observed for a brief period in both the summers of 1964 and 1965. Although the number was small, all adenovirus types 1,6 and 11 were obtained only in winter season of 1965-66, except one type 6 in October, 1966, and some strains of types 2,3 and 5 were also found in this winter season. Two unidentified adenovirus recoveries were made in August of 1964 and in that of 1965.

The majority of enteroviruses occurred in the summer of 1965, and some of them in the summer of 1964 and in the winter of 1965-66.

The Relation of Recovery of Adenoviruses to Age

When the different types of adenovirus are compared with the age of the patients some marked differences are observed, as indicated in Table 5. Of the 676 patients examined,

Table 6. The relation between the types of adenovirus isolated and the defined disease categories, in 470 infants and children.

Disease categories	No. of patients tested	No. of patients having adeno-viruses	Adenovirus							Enterovirus						Total No. of positive patients
										Cox. poliovirus			B	Echo		
			I	II	III	V	VI	XI	?	I	II	III	III	VI	?	
Coryzal syndrome	40	1	—	—	—	—	1	—	—	—	—	—	—	—	5	6
Pharyngeal 〃	98	20	2	2	13	2	—	—	1	—	—	—	—	—	12	31**
P.C.F.*** 〃	23	14*	—	2	10	2	—	—	1	—	—	2	—	—	—	14**
Influenza 〃	4	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
Herpangina 〃	25	0	—	—	—	—	—	—	—	—	—	—	1	—	—	1
L.R.T.I.****	45	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
F.U.O.*****	39	1	—	—	—	1	—	—	—	—	—	—	—	—	1	2
Exanthema	48	6	—	4	—	2	—	—	—	—	1	—	—	—	2	8**
Diarrhea******	98	3	1	1	—	1	—	—	—	—	—	—	—	1	1	5
Pseudocholera infantum	28	3	—	—	—	1	1	1	—	1	—	—	—	—	—	4
Other diseases	22	0	—	—	—	—	—	—	—	—	—	—	—	—	—	0
Total	470	48*	3	9	23	9	2	1	2	1	1	2	1	1	21	71**

* 2 adenovirus strains (type 2 and 5) with a poliovirus type 3 were obtained from one patient.

** 1 or 2 adenovirus strains and an enterovirus were obtained from each of 4 patients.

*** Pharyngo-conjunctival fever **** Lower respiratory tract infection

***** Fever of unknown origin ****** Which except from pseudocholera infantum

107 were infants under 1 year of age, 191 pre-school children of 1-4 years, 172 school children of 5-14 years, and 206 adults of 15 years and over. During the preiod of the survey, adenoviruses were recovered from 12.1% of the infants, 6.3% of the pre-school children, 13.4% of the school children, and none of the adults. All strains of type 3 were found in children between the ages of 1 and 14, and the school children had the highest incidence of this type. Only one strain of adenovirus type 2 was isolated from the school children, and none from the pre-school children, while there were 8 from the infants under 1 year of age. As in type 2, no adenovirus type 5 occurred in the pre-school children and most of them came from the infants. In contrast with adenovirus types 2 and 5, it will be noted that all strains of types 6 and 11 and two of three strains of type 1 were from the pre-school children, 1-4 years, and that both the unidentified adenoviruses were also from these children.

All of enteroviruses were isolated from infants and children, except that only 2 adults shed unidentified enteroviruses of which one was isolated from a man of 17 years of age with the pharyngeal syndrome and the other from that of 31 years with pharyngitis. Six strains identified as poliovirus, coxsackievirus type B3 and echovirus type 6 were recovered from the infants under 1 year.

The Relation of Recovery of Adenoviruses to Disease Category

The disease categories defined and the results of recovery of viruses in infants and children are recorded in Table 6. Among 470 infants and children examined throughout the period of the survey, 190 were in the U. R. T. I. group, 45 in the L. R. T. I. group, 39 in the F. U. O. group, 48 in the exanthema group, and 126 in the diarrhea group. Adenoviruses occurred in 35 of the 190 cases (18.4%) in the U. R. T. I. group, 1 of the 39 (2.6%) in the F. U. O. group, 6 of the 48 (12.5%) in the exanthema group, 6 of the 126 (4.8%) in the diarrhea group, and none of the L. R. T. I. and the other diseases group. In the U.R.T.I. group, adenoviruses were isolated from 60.9% of 23 cases with the P. C. F. syndrome, 20.4% of 98 cases with the pharyngeal syndrome, 2.5% of 40 cases with the coryzal syndrome, and none of 29 cases with the influenza or the herpangina syndrome. Of the 126 cases examined in the diarrhea group, 28 were the patients with pseudocholera infantum and 98 were those with diarrhea associated with U.R.T.I.; adenoviruses were recovered from 10.7% of the former and 3.1% of the latter.

All adenoviruses of type 3 were isolated from the cases with the pharyngeal or the P.C.F. syndrome. There were recoveries of adenovirus types 2 and 5 in the cases with the pharyngeal or the P. C. F. syndrome, the fever of unknown origin, exanthema, or diarrhea. A strain each of adenovirus types 1,6 and 11 were observed in the diarrhea group and the other strains of types 1 and 6 were in the U.R.T.I. group. The two strains of unidentified adenovirus were found in the cases with the pharyngeal or the P. C. F. syndrome.

Approximately half of enterovirus isolations were made from the cases with pharyngeal syndrome, and the others occurred in all the disease categories except the influenza syndrome, the L. R. T. I. group, and the other diseases group.

The Relation of Age and Disease Category in Recovery of Adenoviruses

The influence of age and disease category

Fig. 1. The relation of age and disease category in the patients from whom adenovirus was recovered. Numbers indicate type of the viruses isolated.

Disease categories	0 – 5 Mo.	6 – 11 Mo.	12 – 23 Mo.	2 – 4 Yr.	5 – 9 Yr.	10 – 14 Yr.	15 – 31 Yr.
Coryzal syn.			○	[6] ○	○		
Pharyngeal syn.	[2]	○	[1] ○	[3] ○	[1] [3] [3] ○	[3] ○	○
				? ○	[2] [3] [3] ○	[5] ○	○
				○	[5] [3] [3]	○	
				○	[3] [3]		
					[3] [3] = ○		
P. C. F. syn.	[2] = P 3	[5]	[?]	[3] [3]	[3] [3] [3]	[3] [3]	
		[2] = [5] = P 3		[3] [3]		[3]	
Herpangina syn.		B 3					
F. U. O.		[5]			○		
Exanthema	[2]	[5] [2] ○					
	[2]	[5] [2] = P 2					
		○					
Diarrhea	E 6	[2]		[1] ○	[5]		
Pseudocholera infantum		[5] P 1	[6] [11]				

Key □ =Adenovirus recovery on the first visit

⬚ =Adenovirus recovery on the second visit

○ =Unidentified enterovirus recovery

P =Poliovirus B= Coxsackievirus B E = Echovirus

= =Virus recovery from the same patient

on the patients from whom viruses were recovered may be seen in Fig. 1. Of 23 adenovirus type 3 recoveries 13 were from patients with the pharyngeal syndrome, 11 of whom were school children of 5-9 years. The other types observed in the pharyngeal syndrome were adenovirus types 1,2 and 5, and an unidentified adenovirus and enteroviruses were also found in this syndrome. The remaining adenovirus type 3 recoveries

were made from children with the P. C. F. syndrome whose ages were 2 to 14 years. Three infants under 1 year old with the P.C.F. syndrome had adenovirus type 2 or 5, or poliovirus type 3. This type 2 and an unidentified adenovirus were recovered from the specimens which were collected when the patients revisited during convalescence. In children of 1-7 years with the coryzal syndrome were found an adenovirus type 6, which was obtained from a throat swab on the second visit, and unidentified enteroviruses. Only one coxsackievirus type B3 occurred in an infant aged 9 months with herpangina. There were an adenovirus type 5 and an identified enterovirus recovery in the F.U. O. group ; the former was isolated from an infant and the latter from a school child. 8 cases of exanthema were associated with the recovery of adenovirus type 2 or 5, poliovirus type 2, or unidentified enterovirus ; all patients were under 1 year of age. Adenovirus types 1,2 and 5, echovirus type 6, and unidentified enterovirus were isolated from infants and children with diarrhea associated with U.R.T.I., except pseudocholera infantum ; thier ages were under 9 years. From infants, 6 to 23 months of age, with pseudocholera infantum adenovirus types 5,6 and 11, and poliovirus type 1 were recovered.

The Recovery of Adenoviruses Related to the Type of Specimens and to the Day of Illness Specimens Were Collected

As had already been mentioned in Table 2, eye swabs yielded no virus and all the isolates were obtained from throat and anal specimens. Pharyngeal garglings, all of which were taken from adults of 15 years and over, yielded no adenovirus and yielded only 2 unidentified enteroviruses. Adenoviruses and enteroviruses were isolated more frequently from faeces than from anal swabs.

Table 7 gives the relation of frequency of recovery of viruses from throat and anal specimens in the patients from whom both throat and anal specimen were collected. The patients who revisited hospitals or clinics with no apparent symptom during convalescent phase were regarded here as control group and compared with the patients from whom specimens were collected at the time of illness. Adenoviruses were recovered with equal ease from throat and anal specimens not only in case of the patients with evident symptoms but also in case of those with no apparent symptom. It may be noted that 2 strains of unidentified adenovirus were isolated only from anal specimens and that no enterovirus was recovered from throat specimen alone.

The findings relating the isolation rate of viruses from throat and anal specimens of a total of the 676 patients to the day of illness on which the specimens were collected are presented in Table 8. The day of illness was employed, i. e. if the specimens were taken on the same day as the onset of symptoms, this was the first day of illness ; if the following day, this was the 2nd day. Neither throat nor anal specimens which were collected on the first day of illness yielded adenovirus and the throat specimens collected on the second day gave a low isolation rate (2.7%), although 60% of the 60 throat specimens collected on the first day and 74% of the 183 throat specimens collected on the second day were from the patients with U. R. T. I. as compared with 75% of the 60 anal specimens collected on the first day being from the cases with diarrhea. The day which showed the highest isolation rate for adenovirus from

Table 7. The comparison of recovery of adenoviruses from throat and anal specimens of 350 cases from whom both the specimens were taken.

Type		No. of cases from whom adenovirus was isolated					
		The patients from whom the specimens were collected during acute phase (tested No. =272)			The patients from whom the specimens were collected during convalescence (tested No. =78)		
		Throat only	Throat and anal	Anal only	Throat only	Throat and anal	Anal only
Adenovirus	II	1	1	1	3	1	1
	III	2	12	—	—	—	—
	V	3	1	2	—	—	2
	VI	—	—	1	1	—	—
	?	—	—	1	—	—	1
	Total	6	14	5	4	1	4
Entero-virus	Poliovirus	—	—	2	—	—	1
	?	—	7	5	—	1	2
	Total	0	7	7	0	1	3

Table 8. The isolation rate for adenovirus from throat and anal specimens of a total of the 676 patients examined, according to the day of illness on which the specimens were collected.

Specimens		No. of specimens tested	No. of adenoviruses isolated	Viruses isolated													
				Adenovirus							Enterovirus Poliovirus			C.B		E.		Total
Type	Day of illness			I	II	III	V	VI	XI	?	I	II	III	III	VI	?	
Throat	1	60	0(0.0%)	—	—	—	—	—	—	—	—	—	—	1	—	1	2
	2	183	5(2.7)	1	—	4	—	—	—	—	—	—	—	—	—	10	15
	3	120	9(7.5)	—	2	7	—	—	—	—	—	—	—	—	—	1	10
	4	91	9(9.9)	1	—	5	3	—	—	—	—	—	—	—	—	2	11
	5 - 7	74	6(8.1)	—	—	6	—	—	—	—	—	—	—	—	—	1	7
	8 - 14	71	1(1.4)	—	—	—	1	—	—	—	—	—	—	—	—	—	1
	15 - 21	70	4(5.7)	—	2	—	1	1	—	—	—	—	—	—	—	—	4
	22+	41	2(4.9)	—	2	—	—	—	—	—	—	—	—	—	—	1	3
	Subtotal	710	36(5.1)	2	6	22	5	1	0	0	0	0	0	1	0	16	53
Anal	1	60	0(0.0)	—	—	—	—	—	—	—	1	—	—	—	—	2	3
	2	77	5(6.5)	—	—	2	1	1	—	1	—	—	—	—	—	5	10
	3	54	3(5.6)	—	2	1	—	—	—	—	—	—	—	—	—	2	5
	4	53	6(11.3)	1	—	4	—	—	1	—	—	—	—	—	—	1	7
	5 - 7	75	5(6.7)	—	1	4	—	—	—	—	—	—	—	—	1	2	8
	8 - 14	51	3(5.9)	—	—	2	1	—	—	—	—	—	2	—	—	3	8
	15 - 21	52	6(11.5)	—	1	2	3	—	—	—	—	—	—	—	—	1	7
	22+	39	3(7.7)	—	1	—	1	—	—	1	—	1	—	—	—	1	5
	Subtotal	461	31(6.7)	1	5	15	6	1	1	2	1	1	2	0	1	17	53
Total		1,171	67(5.7)	3	11	37	11	2	1	2	1	1	2	1	1	33	106

throat specimens was the 4th day (9.9%), and the isolation rate was reduced after that day. In case of isolation from anal specimens, adenoviruses were recovered most frequently on the 4th day (11.3%) and on the 15th to 21st day (11.5%). The mean isolation rates of adenovirus for all days irrespective of the day of illness were 5.1% and 6.7% for the 710 throat and the 461 anal specimens, and those for all days greater than 7 were 7/182 (3.8%) and 12/142 (8.5%) for the throat and the anal specimens. Thus, adenoviruses were recovered a little more frequently from anal specimens than from throat specimens in case of all the 676 patients examined, as compared with what were seen in the patients from whom both throat and anal specimen were taken. The throat specimens collected on the 2nd day (5.5%) and the anal specimens collected

Fig. 2-a. The distribution of adenoviruses isolated from throat swabs, according to the day of illness on which the specimens were collected.

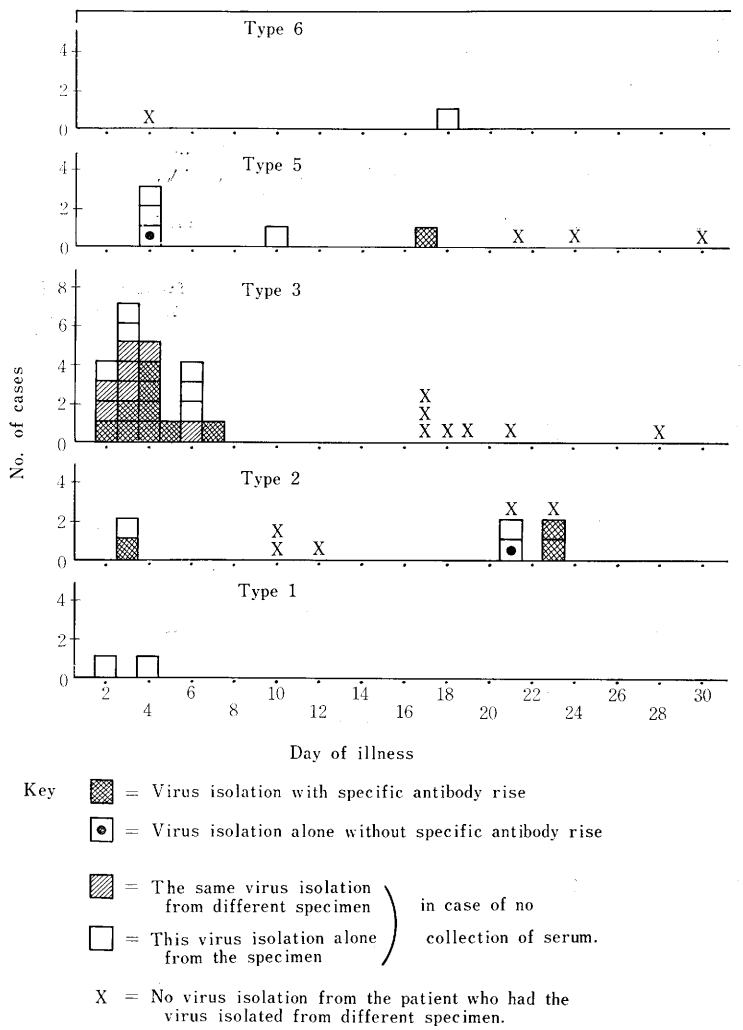
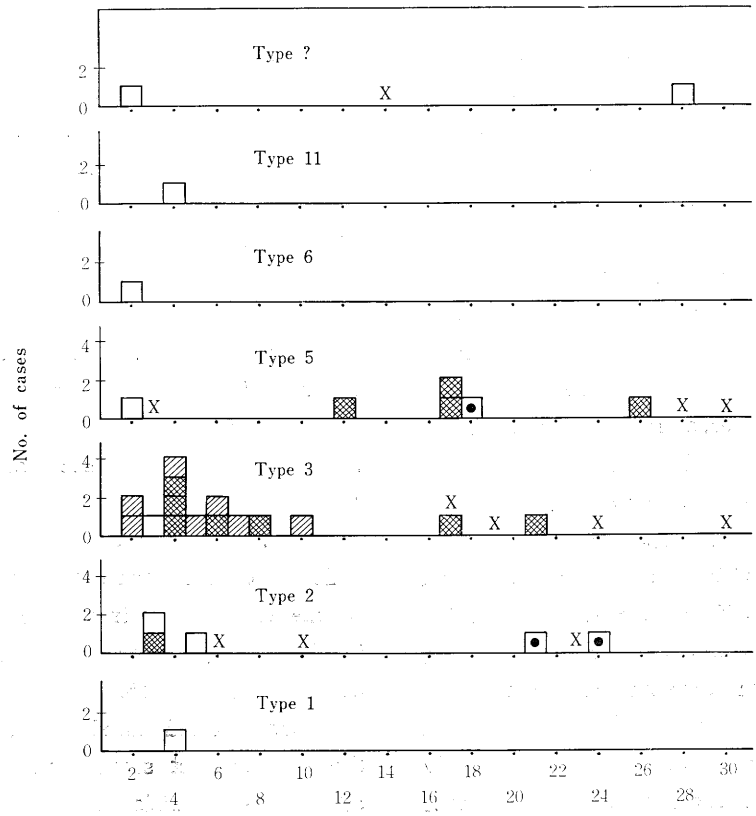


Fig. 2-b. The distribution of adenoviruses isolated from anal swabs and faeces, according to the day of illness on which the specimens were collected.



Key = The same as that of Fig. 2−a.

on the 8th to 14th day (9.8%) yielded enteroviruses most frequently.

The distribution of adenoviruses isolated from throat and anal specimens according to the day of illness the specimens were taken are depicted in Fig. 2-a and Fig. 2-b. All 22 patients who shed adenovirus type 3 from the oropharynx were within 8 days of illness. From 7 of these 22 patients throat specimens were again taken without yielding any viruses during convalescence after the 16th day of illness, and from any of them no specimen taken during the 8th to the 16th day. From the intestine, 13 patients shed adenovirus type 3 by the 11th day of illness, and 2 patients, who had had the same type which was recovered from throat specimens collected during acute phase and showed an antibody rise to the virus isolated, shed on the 17th and the 21st day. Specimens were collected after the 11th day of illness for the first time from 3 of the 9 patients excreting adenovirus type 5; one shed this type from both the

oropharynx and the intestine on the 17th day, the second did from the intestine on both the 12th and the 26th day, and the third did from the intestine only on the 17th day. All these 3 patients produced neutralizing antibody rises in their convalescent serums which were collected after the 25th day of illness. There was one type 5 isolation from a faeces on the 18th day without recovery of this type during acute phase and without a specific antibody rise. From 4 other patients adenovirus type 5 was recovered from throat or anal specimens within 5 days of illness. One patient had this type recovered from a throat swab collected on the 10th day of illness, a poliovirus type 3 from a faeces on the same day, and an adenovirus type 2 from a throat swab on the 21st day. To add to this patient, 4 patients shed adenoviruses of type 2 during the 21st to 24th day of illness, not having this type during acute phase; one excreted this type not only from a throat swab but from a faeces on 21st day without an antibody rise and a poliovirus type 3 from a faeces during acute phase, the second excreted an adenovirus type 2 from a throat swab on the 23rd day with a specific antibody rise and a poliovirus type 2 from a faeces on the same day, the third excreted an adenovirus type 2 from a throat swab alone on the 23rd day with an antibody rise, and the fourth excreted this type from a faeces alone on the 24th day without an antibody rise. Four other patients had type 2 by the 6th day of illness; one of them shed from the oropharynx as well as from the intestine and he showed a rise in antibody. Without recovery during acute phase of illness, a type 6 was recovered from a throat swab and an unidentified adenovirus from a faeces during convalescence.

Antibody Response

The cases confirmed as adenovirus infection by the complement-fixation test are shown in Table 9, by means of age and disease category. Of 136 infants, children and adults from whom blood samples in the acute and the convalescent stages were obtained in addition to materials for virus isolation, 25

Table 9. Adenovirus infections diagnosed by the complement-fixation test*, by age and disease category, June, 1964 to February, 1966.

Disease category	Age				Total positive	Total tested	Average
	0-11 Mo.	1-4 Yr.	5-14 Yr.	15-64 Yr.			
Coryzal syndrome	—	—	—	4	4	32	12.5
Pharyngeal syndrome	—	1	6	1	8	40	20.0
P.C.F. syndrome	1	2	2	—	5	13	38.5
L.R.T.I.	—	1	—	—	1	12	8.3
F.U.O.	—	1	1	—	2	15	13.3
Exanthema	2	—	1	—	3	11	27.3
Diarrhea	—	—	2	—	2	5	40.0
Other diseases	—	—	—	—	0	8	0
Total positive	3	5	12	5	25	⋮	⋮
Total tested	17	31	51	37	.....	136	⋮
Average	17.6	16.1	23.5	13.5	.....	.....	18.4

* Against the pooled antigen of types 3 and 5 isolated

Table 10. The CF and the neutralizing antibody response of the patients from whom adenovirus was recovered from throat or anal specimen, or both.

Type of adeno-virus	Adenovirus recovered from								
	Throat specimen only			Throat and anal specimen			Anal specimen only		
	No.	Antibody rise*		No.	Antibody rise		No.	Antibody rise	
		CF**	NT***		CF	NT		CF	NT
		%	%		%	%		%	%
II	3	0	67	2	50	50	2	0	0
III	2	100	100	6	100	100	0	—	—
V	1	0	0	1	100	100	3	33	33
Total	6	33	67	9	89	89	5	20	20

* A fourfold or greater rise
** Against the pooled antigen of types 3 and 5
*** Against the virus isolated

(18.4%)showed a fourfold or greater antibody rise to the CF antigen pooled types 3 and 5 isolated. 11 of them occurred in patients from whom adenovirus was isolated and 14 in those from whom no virus was recovered. The percentage of patients with a fourfold or greater CF antibody response in the different age groups was 17.6% in the infants under 1 year of age, 16.1% in the pre-school children between 1 and 4 years, and 23.5% in the school children of 5-14 years. As compaired with no recovery of adenovirus from the adults over 14 years old and only one recovery from the children with the coryzal syndrome, 5 adults (13.5%) were positive by the CFT; 4 of them were suffering from the coryzal syndrome and the other from the pharyngeal syndrome.

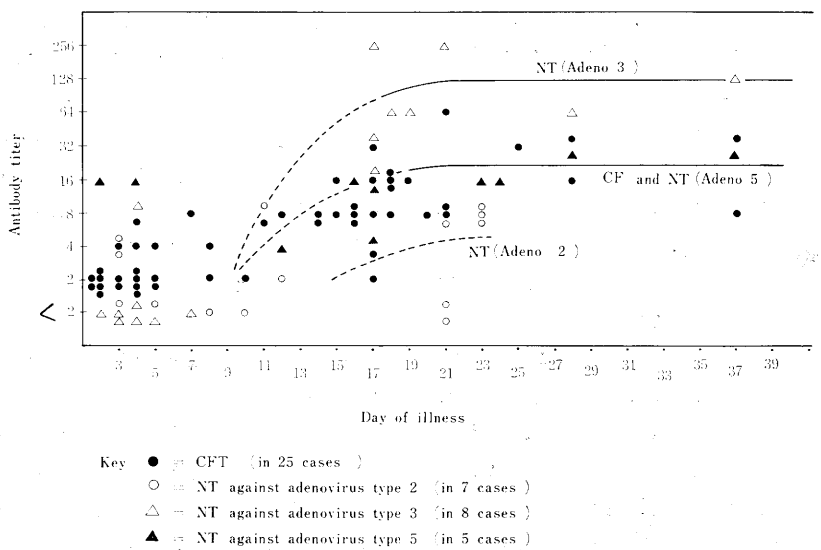
Seven children with the pharyngeal syndrome, from 5 of whom adenovirus type 3 was isolated, showed a CF antibody rise; 6 of them were the school children. In infants and children with the PCF syndrome there were 5 with a rise in antibody titer; all but one were associated with the recovery of adenovirus. Although no adenovirus was recovered from the patients in the L. R. T. I. group, only one child with bronchitis produced an antibody rise. Of 7 patients in the F. U. O., the exanthema and the diarrhea group, 2 were infants with exanthema from whom adenovirus was recovered and the others were children from whom no virus was isolated.

Paired serums were obtained from only 22 of the 48 patients excreting adenoviruses. A fourfold or greater rise both in complement fixing and in neutralizing antibody titer occurred in 9 of the 22 cases, it in complement fixing antibody titer alone occurred in 2, and it in neutralizing antibody titer alone occurred in 4. Thus, of the 22 patients 11 showed a fourfold or greater rise in CF antibody and 13 showed it in neutralizing antibody. From the case who shed adenovirus type 2 and 5 with poliovirus type 3 a pair of sera was taken; no complement fixing antibody rise was detected and a neutralizing antibody response to each type isolated could not be demonstrated from want of an amount of serum. A serum pair which was obtained

from one of the 2 cases excreting unidentified type gave a twofold rise in each of complement fixing and neutralizing antibody. Excluding these 2 patients, in Table 10 the CF and the neutralizing antibody response of 20 cases from whom adenoviruses of types 2, 3 and 5 were recovered are analyzed to show the response according to each of the types and to indicate the response according to whether adenovirus was recovered from a throat or an anal specimen, or both. A fourfold or greater rise in CF and neutralizing antibody each occurred in 89% of the patients when the same virus was isolated from both throat and anal specimen. In contrast to this, only 33% of patients in whom adenovirus was recovered from the throat specimen alone produced a CF antibody rise and only 20% of those in whom adenovirus was recovered from the anal specimen alone did it. Neutralizing antibody responses were found in 67% of the cases shedding adenovirus from the throat alone and 20% of those shedding it from the intestine alone. Adenovirus type 3 isolations were always accompanied by both CF and neutralizing antibody rise, but only 1/7 (14%) of the cases from whom type 2 was recovered developed a CF antibody response and 3/7 (43%) of those a neutralizing antibody response. There were a CF and a neutralizing antibody rise in 40% of 5 patients from whom the recovery of type 5 was made.

The CF and the neutralizing antibody response to adenovirus infections according to the day of illness are shown in Fig. 3. A curved line for CF antibody response to adenovirus was drawn by making an entry of the antibody titers in the acute and the convalescent serums of 25 cases who showed a fourfold or greater CF antibody rise. As will be seen, the CF antibody began to rise from about the 8th to 10th day of illness and got to the peak by the 20th day. Neutralizing antibody titers to the isolated viruses in the acute and the convalescent serums of the

Fig. 3. The CF and the neutralizing antibody response to adenovirus infections, according to the day of illness.



cases from whom adenovirus type 2, 3 or 5 was recovered were filled up, and the curves for neutralizing antibody response were drawn, though the number was very small. A curve for neutralizing antibody response to adenovirus type 5 was the same curve as that for the CF antibody response, and a neutralizing antibody to type 3 seemed not only to begin to increase but also to reach the highest at the same time as the CF antibody. The patients who shed adenovirus type 2 had very low neutralizing antibody titers to this isolated virus in the convalescent serums.

Clinical Description of Adenovirus Infection

Table 11 gives the frequency distribution of various signs and symptoms in each of adenovirus infections diagnosed by the recovery of adenovirus and by the CFT. These signs and symptoms were obtained with consultations and interrogations, but some of them could not get answers from the infants and babies. Each percentage of various symptoms of the patients from whom adenovirus was recovered was almost all the same as that of the patients who were confirmed by the CFT. Constitutional symptoms such as fever, headache, anorexia and fatigue and respiratory symptoms such as redness of the pharynx, etc. were present at a high rate. Conjunctival injection was shown in about 35% of the patients, and abdominal symptoms such as vomiting and diarrhea in approximately a quarter. Cervical lymphadenophathy and skin rash were not common, but 15% of the patients had the former and 12% of those had the latter.

In addition to 5 patients with exanthema from whom an adenovirus was recovered

Table 11. The frequency distribution of signs and symptoms in each of adenovirus infections by the isolation and by the CFT.

Signs aud symptoms	Type of adenoviruses isolated							CFT
	I	II	III	V	VI	XI	Total	
Fever	3/3	5/6	21/21	8/8	1/2	1/1	39/41(95)	22/25(88)
Headache	1/2	1/3	17/19	1/5	0/1	—	20/30(67)	14/21(67)
Anorexia	1/3	1/6	15/21	2/8	2/2	0/1	21/41(51)	13/25(52)
Fatigue or displeasure	1/2	1/5	15/21	4/8	1/1	0/1	22/38(58)	14/25(56)
General achings	—	—	2/19	1/5	—	—	3/24(13)	0/21(0)
Conjunctival injection	0/3	1/6	10/21	4/8	0/2	0/1	15/41(37)	8/25(32)
Nasal obstruction and discharge	1/3	2/6	7/21	5/8	2/2	0/1	17/41(41)	9/25(36)
Sore throat	0/2	0/3	11/19	0/5	0/1	—	11/30(37)	8/21(38)
Redness of the pharynx	1/3	4/6	18/21	4/8	0/2	0/1	27/41(66)	20/25(80)
Swelling of the tonsils	1/3	2/6	15/21	2/8	0/2	0/1	20/41(49)	10/25(40)
Cough	1/3	2/6	7/21	4/8	0/2	1/1	15/41(37)	10/25(40)
Nausea and vomiting	2/3	2/6	7/21	2/8	1/2	1/1	15/41(37)	6/25(24)
Diarrhea	1/3	2/6	3/21	2/8	1/2	1/1	10/41(24)	4/25(16)
Cervical lymphadenopathy	0/3	1/6	3/21	2/8	0/2	0/1	6/41(15)	4/25(16)
Skin rush	0/3	3/6	0/21	2/8	0/2	0/1	5/41(12)	3/25(12)

Notes : Numerator=positive number. Denominator=total number of cases reported.
( )=average.

ni this table, an infant with skin rash was excreting adenovirus type 2 and poliovirus type 2 as had been already mentioned in Table 3 and in Fig. 1. All of them were admitted in the Sackling Asylum. The case reports of them are as follows.

Case 1. K. Azuma, 8 months old, male, had a temperature of 38.5°C and the coryzal syndrome with conjunctivitis on August 10, 1964, and since then a temperature of approximately 38.5°C lasted until August 22. On August 26, micropapular rash just like scarlet fever developed on the whole body surface except the face. It disappeared leaving no desquamation and pigmentation on the day after the following day. On August 30, he had again a fever of 38.5°C and kept a temperature of about 38.0°C up to September 19. Throat and conjunctival swabs and faeces were collected on August 26 and on September 6, and investigated for adenovirus. There was an adenovirus type 5 recovery from the faeces only collected on August 26. An antibody rise was detected in serum taken on the 28th day of illness.

Case 2. I. Ueki, 9 months old, male. His illness started on August 15, 1964, with a temperature of 38.1°C, the pharyngeal syndrome, conjunctival injection and cervical lymphadenopathy. His fever was gone on August 18. On August 21 he had again a fever of 38.1°C, and his temperature lasted until August 24. On August 25, rash developed on the whole body surface except the lower limbs. It was micropapular appearance just like scarlet fever and disappeared a few days later, leaving no pigmentation. On September 10 and 11, he had again a fever with conjunctivitis. Although no virus was obtained from specimens which were collected on August 26, adenovirus type 2 was recovered from a throat swab collected on September 6 and poliovirus type 2 from a faeces on the same day. He produced an antibody rise to the adenovirus type 2 alone.

Case 3. S. Matsukawa, 7 months old, male, had a fever of 37.4°C with the coryzal syndrome on September 6, 1965, and the temperature fell down associated with conjunctival injection on the following day. On September 12, he showed scarlet fever-like micropapular exanthema on the whole body surface except the face with hepatic and splenic enlargement. The rash lasted with them until September 21, and on the next day he had a fever of 39.2°C. He shed adenovirus type 5 from both the oropharynx and the intestine on the 17th day of illness and was positive by the neutralization test and the CFT.

Case 4. H. Tanio, 5 months old, female. She began to be sick on September 20, 1965, with a fever of 37.8°C, coughs, rhinorrhea and redness of the pharynx. On the next day, she had a temperature of 39.3°C. Three days after the onset of illness, her fever was gone. On the following day, she showed skin rash just similar to that of scarlet fever or rubella on the whole body surface especially on the abdominal side of the trunk. The rash disappeared several days later, leaving no desquamation and pigmentation. Type 2 adenovirus recovery was made from both throat swab and faeces collected on the 3rd day of illness, and a specific antibody rise was detected.

Case 5. H. Matsumoto, 11 months old, male. He had a temperature of 37.8°C with conjunctivitis on September 20, 1965. On the next day rash appeared mainly on the abdominal side of the trunk with a fever of 37.9°C, and then it spread on the whole body suface with-

Table 12. The efficacy of isolation related to the method of storage of the specimens from the time of collection before they were transported to the laboratory.

		Specimens which were kept in each of the hospitals and clinics before they were transported to the laboratory							
		at 4°C				at room temperature			
		less than 6 hours	1-2 days	3-5 days	more than 5 days	less than 6 hours	1-2 days	3-5 days	more than 5 days
No. of materials collected		315	371	325	109	31	49	56	32
No. of virus recovery	Adenovirus	15(4.8)	16(4.3)	15(4.6)	13(11.9)	2(6.5)	4(8.2)	2(3.6)	0(−)
	Enterovirus	7(2.2)	8(2.2)	11(3.4)	7(6.4)	1(3.2)	3(6.1)	2(3.6)	0(−)

Note : ( ) =The rate of isolation

Fig. 4-a. The relation between the time required for the first detection of the CPE and the method of storage of the throat specimens from the collection before the transportation to the laboratory. Numbers indicate type of adenovirus isolated.

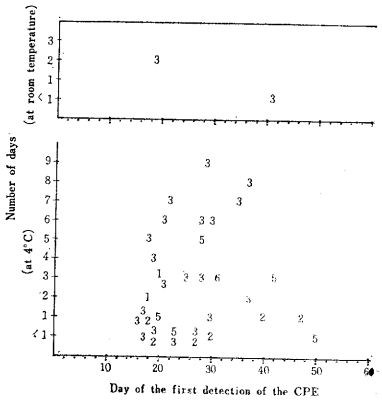
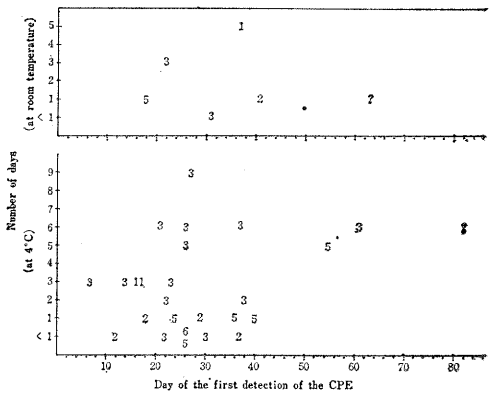


Fig. 4-b. The relation between the time required for the first detection of the CPE and the method of storage of the anal specimens from the collection before the transportation to the laboratory. Numbers indicate type of adenovirus isolated.



out fever and lasted until September 26. The rash resembled that of scarlet fever or rubella. He had a fever of 38.4°C with rhinorrhea and redness of the pharynx on October 7, and had again a fever of 38.8°C with coughs and vomiting on October 13. Throat swabs and faeces were taken from him on September 22, on September 27, and on October13. Adenovirus type 2 was obtained only from the throat swab on October 13, and he showed a specific antibody rise to the virus isolated.

Case 6. M. Muranaka, 5 months old, male. His illness started on September 22, 1965, with a fever of 39.5°C, coughs, rhinorrhea, vomiting and redness of the pharynx, and these symptoms were recognized on the next day. On September 24, he had a normal temperature and had exanthema resembling that of Cases 4 and 5 on the whole body surface, excreting adenovirus type 2

from the throat. The rash disappeared a few days later leaving no pigmentation. He showed a twofold neutralizing antibody rise, but showed no CF antibody response.

The Relation Between Recovery of Viruses and Method of Storage of the Specimens Before Transported to the Laboratory

In each of the hospitals and clinics from the time of collection before they were transported to the laboratory, only 315 (24.5%) of the total specimens were kept at 4°C less than 6 hours, 805 (62.5%) at 4°C more than 24 hours, and the others (13.0%) at room temperature a few hours or several days. As can be seen in Table 12, adenoviruses or enteroviruses were recovered with equal ease from each of the specimens, whether they were kept at 4°C or at room temperature for less than 6 hours or for 1 to 5 days. When the specimens were kept at 4°C more than 5 days, these specimens yielded not only adenoviruses but enteroviruses approximately twice as frequently as the specimens which were kept 5 days and less did. In case that the specimens were kept at room temperature more than 5 days, these specimens yielded no virus.

The time required for the first detection of the CPE related to the method of storage of the specimens before transported to the laboratory is given in Fig. 4-a and 4-b. It was observed that there was little influence of longer than 6 hours storage at 4°C or at room temperature on the time required for the first detection of the CPE. With all of the adenoviruses, 50% of the virus-positive throat specimens showed a 3+ CPE in HeLa cell culture by the 25th day after inoculation, and 90% did it by the 40th to 41st day. CPE was produced a little later in tissue cultures inoculated with anal specimens than with throat specimens. Adenoviruses of types 1,2,3,5,6, and 11 seemed to produce CPE at the same rate, but only 2 unidentified adenoviruses produced CPE more slowly than those adenoviruses.

Discussion

Though the method of collecting specimens was not proper because of only 24.5% of the total specimens spending less than 6 hours at 4°C and moreover 13.0% of those spending a few hours or several days at room temperature in each of the hospitals and clinics from the time of collection before they were transported to the laboratory, it is worth noticing that 49 adenovirus strains were isolated from 48 patients (7.1%) out of the 676 examined in the practice. If enteroviruses are included, 73 (10.8%) of the 676 patients were infected with adenovirus or enterovirus. HeLa cells alone were employed throughout the study, and the total incubation period in each of the negative specimens was at least a total of 80 days through three passages, cultures being maintained in YLH containing usually 3 to 4 per cent bovine serum. If the specimens had been inoculated into cell cultures at the bedside (Holzel *et al.*, 1963[33] ; Higgins *et al.*, 1964[27]) or frozen in dry ice within 30 minutes after collection, and if embryo calf or chicken serum had been used in the maintenance medium because of not only bovine serum but even rabbit and calf sera being often inhibitory to viruses found in the throat (Hamre, 1963[24]), more viruses would have been isolated. Through relatively high stability of adenovirus such as retaining

infectivity at 4°C for 2 months or more at a pH of 6.5-7.5 or at room temperature for 14 days (Ginsberg, 1956[19]), it is natural results that adenoviruses were recovered with equal ease from each of the specimens, whether they were kept at 4°C or at room temperature for less than 6 hours or for 1 to 5 days, and that there was little influence of longer than 6 hours storage of specimens at 4°C or at room temperature on the time required for the first detection of the CPE. When the specimens were kept more than 5 days at 4°C or at room temperature, those at 4°C yielded adenoviruses approximately twice as frequently as the specimens kept for 5 days and less, and those at room temperature yielded no virus. But this may be the result from the smallness in number. Provided adenoviruses and some of enteroviruses are going to be isolated, these kinds of specimens must be able to be used for virus isolation since these findings were also observed in recoveries of enteroviruses, which gave a relatively low isolation rate. The findings that 90% of the adenovirus-positive throat specimens produced a CPE by the 40th to 41st day after inoculation as found in this study is not so successful as the finding reported by Vargosko *et al.* (1965)[56], who found that 90% of those showed it by the 15th day in continuous human cell cultures. This might be accounted for with the maintenance medium containing bovine serum, with the day for the first detection being decided when the cultures showed a 3+ CPE in this study, and not with the delay in the inoculation of the specimens reducing the amount of virus. CPE was observed to be produced a little later in tissue cultures inoculated with anal materials than with throat materials. This suggests that there was some viral inhibitor in anal specimens or that anal specimens contained slightly smaller amounts of virus than did throat specimens. However, it is considered not to be due to these suggestions but to be due to their character itself producing very little cellular change that more than 60 days were required to detect 2 unidentified adenoviruses.

The 49 adenovirus strains were principally type 3, the so-called pathogenic type, which appeared most significantly in a sharp outbreak in the summer of 1965 (19 of the 23 isolates), and types 2 and 5, the endemic types, which occurred predominantly for a short time in each of the summers of 1964 and 1965. The other endemic types of adenovirus, types 1,6 and 11, and 2 unidentified types were also recovered. These adenovirus strains were obtained from 5.1% of the 710 throat specimens and from 6.7% of the 461 anal specimens. Eye swabs yielded no virus and this might be due to an improper method of swabbing conjunctiva in most of the cases. Considering that adenoviruses had been recovered with equal ease from throat washings and throat swabs (Rosenbaum *et al.*, 1959[50]; Bell *et al.*, 1955[2])and that adults never shed adenovirus even from the intestine in this study, it must have been correct that not throat swabs but throat washings were collected from adults, even though all pharyngeal garglings yielded no adenovirus in the present study. Recoveries of adenoviruses with equal ease from throat and anal specimens in case of the patients from whom both the specimens were collected is not in accordance with the findings of the other studies (Moffet and Cramblett, 1962[44]; Vargosko *et al.*, 1965[56]), in that adenoviruses were isolated approximately two to five

times as frequently from anal as from throat specimens. This might be due to the smaller number of the cases from whom both throat and anal specimen were collected in this study.

It is generally said that adenoviruses are present in the nasopharynx or oropharynx for a brief period early during human infection and then they persist for a longer interval either in gastrointestinal mucosa or lymphoid tissue and that therefore there is a greater chance of isolating the virus if the pharynx of the patient is swabbed in the early stage of the illness. Couch *et al.* (1963)[5] have suggested through the studies with volunteers that fecal excretion of adenovirus is no effective means for transmission of the agent to other adults, but it is very profitable for prevention of an epidemic to know the viral excretion period from the oropharynx and the intestinal tract, and it is also very useful for virological diagnosis to know actually the optimal time for taking specimens. Although specimens were not obtained from the same patients with a few exception, these subjects must be inferred from the frequency distribution of viruses isolated on the day of illness on which the specimens were collected. It is interesting to note that not only the anal specimens but the throat specimens which were collected on the first day yielded no adenovirus. In such an early stage of an infection as the symptoms were general rather than local, it can not be denied that illness not due to viruses may have been included in the study or that the amount of virus enough to be isolated may not have become sufficient for detection. These findings therefore suggest that throat specimens collected on such a day as the onset of symptoms are not yet worthy of virus isolation as well as anal one. The day showing the highest isolation rate for adenovirus from throat specimens was the 4th day of illness and that from anal specimens was the 4th day and the 15th to 21st day, suggesting that adenovirus is excreted in a relatively early stage from the throat and for a fairly long time from the intestine. For both adenovirus and enterovirus, the optimal time for taking throat specimens appears to be the 2nd to 4th day and that for taking anal specimens to be from the 2nd day until about 21st day, since enteroviruses were isolated from throat specimens on the 2nd day and from anal specimens on the 8th to 14th day at the highest rate and on the 2nd day at a rather high rate. These findings were similar to that found by Higgins *et al.* (1963[25] and 1964[26]). Adenovirus type 3 had turned out to be shed from the oropharynx within 8 days of illness and not to be shed after the 16th day at the longest, in contrast with being shed from the intestine until approximately the 21st day. However, adenovirus type 2 was excreted not only from the intestine but from the throat longer than type 3 from the intestine, and type 5 excreted shorter from the throat and longer from the intestine than type 2. These facts are responsible for the isolation rate of 3.8% and 8.5% for adenovirus from the throat and the anal specimens which were collected on the 8th day and later. Since the onset of the illness was sometimes difficult to be determined, some of the late isolations may be due to a new illness.

The occurrence of multiple virus isolations from 4 patients may be due either to simultaneous or to sequential infection with 2 or more viruses from some time before the first collection till the second. Many other students have found double virus infections.

Two antigenic types of adenovirus have been isolated from each individual patient (Bell, 1965[3]; Vargosko *et al.*, 1965[56]), and recoveries of an adenovirus and an enterovirus have simultaneously occurred (Bell, 1965[3]). Additionally, simultaneous recoveries of an adenovirus and a respiratory syncytial virus and those of an adenovirus and a myxovirus have been recorded (Hilleman *et al.*, 1962[28]; Urguhart *et al.*, 1965[55]). An adenovirus and an enterovirus recovery from a throat and an anal material collected on the same day in each of 3 of the 4 patients in this study indicate that an adenovirus infection does not exclude an enterovirus one which usually takes place in another part of the body. Two instances (case No. 31 and 34 in Table 3) of the 4 patients may have experienced sequential infection with the agents. Because they were again in a fever before and after the second specimens were taken and they showed a specific antibody rise against only one of the two viruses isolated. In the other two there was no serological diagnosis; in one case because of taking no serum in convalescence and in the other from want of an amount of serum. These two, from one of whom 2 adenoviruses of types 2 and 5 and a poliovirus of type 3 were recovered, had never manifested any symptoms for 1 to 2 months before and after the onset of symptoms. Therefore, they could not make clear whether the occurrence of the multiple virus isolations results from simultaneous or sequential infection with the agents.

When the isolated adenoviruses were compared with the age of the patients, it is observed that school children of 5 to 14 years gave the highest isolation rate of 13.4% and that there was no recovery of adenovirus in adults of 15 years and over. It is interesting that 5 of the 37 adults (13.5%) were confirmed as adenovirus infections only by a rise in CF antibody titer in comparison with no adenovirus isolation from any specimens of 206 adults, suggesting that infections with this virus do fairly frequently occur in this age group. This no isolation in the adults is therefore assumed to originate in too small quantity of virus they excreted to be isolated. However, very few adenovirus infections in adults is thought to exist in Japan, considering that 4 of the 5 CF-positive patients were suffering from the coryzal syndrome, that in Japan very few adenovirus recoveries have been made from adults, and that in other countries the isolation rate of 0 to 4% for adenovirus from adults with acute respiratory disease has been reported by many investigators (Evans, 1958[11]; Grayston *et al.*, 1958[21]; Grieble *et al.*, 1958[22]; Holland *et al.*, 1960[32]; Evans *et al.*, 1961[12]; Hilleman *et al.*, 1962[28]; Higgins *et al.*, 1964[26]).

Comparing the different types with the age, there was significant difference with type 3 and type 2 or 5 among different age groups in the children. Having regard to the epidemic and the latent type, it is a matter of course that most of the type 3 strains were recovered from the school children and that the types 2 and 5 were mainly isolated from the infants. Although the number was small, pre-school children of 1 to 4 years yielded all strains except one of the other latent types of adenovirus, types 1, 6 and 11, and both of the unidentified types.

Among the 22 individuals excreting adenovirus from whom the paired serums were obtained, only 9 produced a fourfold or greater rise both in CF antibody and in neutralizing antibody, 2 in CF antibody alone, and 4 in neutralizing antibody alone. One of the

2 cases producing a fourfold or greater CF antibody alone showed a twofold rise neutralizing antibody, but the other did not. Of the 4 cases producing a fourfold or greater neutralizing antibody alone, 3 who had CF antibody titers of 1:4 or less in their acute serums showed a twofold rise in CF antibody and the other who had a CF antibody titer of 1:16 in his acute serum did not. Among 7 cases who showed a fourfold or greater rise neither in CF antibody nor in neutralizing antibody, one showed a twofold rise in both CF and neutralizing antibody, another in CF antibody alone, and other two in neutralizing antibody alone. These findings should be sufficiently interpreted, seeing that clinical or epidemiological study is usually based on the cross-reactive complement-fixation test. They may have been due to several factors that insufficient time was allowed between acute and convalescent serums in some cases to occur, that the time of the initial collection of sera was sometimes too late to demonstrate an antibody rise, and that some group reactive antibody existing in acute serum as a result of infection with a different serotype in the recent past was apt to mask a rise in antibody due to the present infection, vainly dropping a hint that a neutralizing antibody may be produced on some occasion in an earlier stage of infection than a CF antibody.

If the time of collection of sera was supposed to be appropriate for the test in this study, among the 22 cases 13(59.1%) who had a significant neutralizing antibody rise would be adenovirus infections and 40.9% adenovirus carriers, since a fourfold or greater rise in antibody is regarded as virus infection whether virus has been successfully isolated or not. On the other hand, 11(50.0%) of the 22 cases who excreted adenovirus and 14 (12.3%) of 114 cases who had no virus were accompanied with a fourfold or greater rise in CF antibody, and, therefore, 25 (18.4%) of a total of 136 patients from whom the pair of sera was obtained were confirmed by the CFT. In the different age groups, the school children of the ages of 5 to 14, who yielded the highest isolation rate of adenovirus, showed the highest percentage (23.5%) of cross-reactive CF antibody rises as well, and the adults, who yielded no adenovirus, showed the lowest percentage (13.5%). In the light of findings of Urguhart *et al.* (1965)[55] it is not unexpected that 89% of the cases shedding the same strain both from the throat and from the intestine and only 20% of those shedding from the intestine alone gave a fourfold or greater antibody rise. Therefore, when the same virus has been isolated from both throat and anal specimen, almost all the cases should be regarded as infection and not carriage, even if serological tests are not carried out. However these findings in this study is not in accordance with that of Vargosko *et al.* (1965)[56], who found that a fourfold or greater rise in CF antibody was detected in only 37% of 43 cases from whom adenovirus type 1,2,3,5 or 7 was recovered and in whom the same strain was recovered from both throat and anal swab. The findings of a neutralizing antibody rise in 100% of the patients excreting adenovirus type 3 and in approximately 40% of those excreting type 2 or 5 indicate that type 3 is always associated with illness but types 2 and 5, the so-called latent type, are not always, though often.

Since a neutralizing antibody against type 3 or 5 and a CF antibody seemed to begin to increase from about the 8th to 10th day of illness and to get to the peak by the 20th day as seen in Fig.3, the serum pairs taken within

7 days of illness for the acute serum and those taken after the 20th day for the convalescent serum will be enough for serological tests. Moffet *et al.* (1962)[44] suggest that an adenovirus is less likely to be recovered in early infancy, when transplacentally acquired serum neutralizing antibodies may still be present. Vargosko *et al.* (1965)[56] have found that the highest percentage (31%) of CF antibody response occurred in infants in the age group of 7 to 12 months when maternal antibodies were usually undetectable, and indicated that this is the time of life when primary infection is most likely to occur, and, therefore, virus isolation is most likely to be associated with acute infection. In the present study, there were very low neutralizing antibody titers to the isolated virus in the convalescent serums of 7 infants under 1 year of age from whom adenovirus type 2 was isolated, though 4 of them ranged the ages of 6 to 11 months. This is inferred to result from the possibility that some cases were due to illnesses infected with the different viruses and due to a new illness infected with this type towards the time of collection of the convalescent serum, because this type was isolated only from the materials which were collected during convalescence in 4 of the cases, it is, indeed, suggested either that this type has very small ability to produce a detectable antibody in patients or that the infants have very small ability to produce a detectable antibody against this type.

It is very important to know that a virus was associated not only with illness but with a particular clinical syndrome. Adenovirus infections have been found in 5 to 14 per cent of infants and children suffering from upper respiratory illness and in 2 to 12 per cent of those suffering from lower respiratory illness (Morrison *et al.*, 1957[45]; Gardner *et al.*, 1960[18]; Holland *et al.*, 1960[32]; Kapikian *et al.*, 1960[40]; Hilleman *et al.*, 1962[28]; Moffet and Cramblett, 1962[44]; Clarke *et al.*, 1964[4]; Higgins *et al.*, 1964[26][27]; Urguhart *et al.*, 1965[55]). In the present study 36 strains of adenovirus were isolated from 35 cases of 190 infants and children with upper respiratory symptoms, giving an isolation rate of 18.9%. Among the 35 cases 20 were suffering from pharyngeal symptoms and 14 from pharyngo-conjunctival fever. Ten of the 14 cases with PCF were associated with the recovery of adenovirus type 3, and an association of PCF with this type has been well documented (Bell *et al.*, 1955[2]; Huebner *et al.*, 1958[34]; Kaji *et al.*, 1961[37][38]). However, no recovery of adenovirus type 3 occurred in 4 cases with PCF; two cases under one year of age excreted 2 or 3 strains among types 2 and 5 of adenovirus and type 3 of poliovirus, and the others excreted type 5 or unidentified type of adenovirus. On the other hand, there was no recovery of adenovirus in 45 infants and children suffering from lower respiratory illness.

Other investigators (Hirayama, 1959[31]; Kamiya *et al.*, 1961[39]; Takatsu, 1961[54]) have found that adenovirus types 1,2,5,6, and poliovirus type 3 were isolated from anal specimens of infants with pseudocholera infantum, which Ito(1910)[35] called a disease with good prognosis characterized by vomiting and diarrhea with white faeces in infants chiefly during the weanling period. The disease, which was explained by Enjoji(1958)[10] to be a clinical syndrome as the result of a nonspecific constitutional reaction associated with cold weather with or without infection of bacteria or virus, occurs only in the season

from late fall to early winter. In the present study 6 strains of adenovirus were found in 126 infants and children with diarrhea, with an isolation rate of 4.8%. Three of them came from the cases under 2 years of age with white faeces during the winter season, and were identified as adenovirus types 5,6 and 11. There were some recoveries of these types from anal specimens of infants without diarrhea in this study, and other observations indicate that adenoviruses are frequently recovered from the anal specimens of apparently healthy infants (Joncas and Pavilanis, 1960[36]; Vorgosko *et al.*, 1965[56]) and that any adenovirus isolated is not associated with diarrhea although 76% of the total of isolations were from the anal swabs only (Moffet and Cramblett. 1962[44]). Nevertheless, as a proportion of pseudocholera infantum was indicated by Fukumi (1960)[17] to be caused by adenovirus, so the data from this study indicate that some types of adenovirus give a rise to a proportion of diarrhea, especially of pseudocholera infantum which occurs in the winter season.

Several cases of recoveries of adenovirus types 2, 3, 4 and 7 have been reported in which rashes resembling measles, rubella, scarlet fever, exanthema subitum, urticaria or roseola infantum were observed with fever, pharyngitis or conjunctivitis (Neva and Enders, 1954[46]; Fukumi *et al.*, 1957[15]; Fukumi *et al.*, 1958[16]; Fujii, 1961[13]; Gutekunst and Heggie, 1961[23]). In this study the recovery of adenovirus types 2 and 5 was made from 6 infants under 1 year with skin rash. It is interesting to note that the rash which was seen in each of 3 infants excreting adenovirus type 2 was similar to that of scarlet fever or rubella and developed on the whole body surface especially on the abdominal side of the trunk. On the other hand, recovery of adenovirus type 5 which occurred in each of the summers of 1964 and 1965 in 2 infants was associated with micropapular rash just like scarlet fever on the whole body surface except the face. An infant from whom adenovirus type 2 and poliovirus type 2 were obtained showed exanthema resembling scarlet fever on the whole body surface except the lower limbs. All of their rashes disappeared in a few days, leaving no pigmentation. It may be concluded from these findings that a proportion of skin rashes is due to the adenovirus infections and that a certain type of adenovirus shows a certain appearance of rash, but the cases will be too few to explain the skin rashes originating in infections of adenovirus.

Summary

Aetiological and clinical studies on adenovirus infections were carried out with the total of 676 patients with acute respiratory illnesses, diarrhea or skin rash in Nagasaki, Japan. The results are as follows:

(1) Forty-nine adenovirus strains were isolated from 48 of 470 infants and children; they contained three of type 1, nine of type 2, twenty-three of type 3, nine of type 5, two of type 6, one of type 11, and two of unidentified type.

(2) Conjunctival swabs yielded no virus and all the isolates were obtained from throat and anal specimens; adenoviruses were isolated a little more frequently from anal specimens (6.7%) than from throat specimens (5.1%).

(3) Type 3 occurred mainly in school

children, types 2 and 5 in infants, and the other types in pre-school children.

(4) All the types except 11 were recovered from the patients with upper respiratory illnesses, types 5,6 and 11 from those with pseudocholera infantum, and types 2 and 5 from those with rash resembling scarlet fever or rubella.

(5) The optimal time for taking throat specimens for adenovirus isolation was the 4th day of illness, and that for taking anal specimens was the 4th day up to about 21st day.

(6) Twenty-five of 136 patients (18.4%) were confirmed as adenovirus infection by the CFT.

(7) The delay in the delivery of specimens to the laboratory had little influence on the isolation rate and on the time required for the first detection of the CPE.

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長崎市におけるアデノウイルス感染症の病因的、臨床的研究（1964—1966）

二ッ木浩一

長崎大学熱帯医学研究所ウイルス学部（主任：福見秀雄教授）

摘　　要

1964年6月から1966年2月に至る期間，長崎市内の公私立病院・医院・乳児院等12施設の外来および入院患者計676名についてアデノウイルス感染症の病因的，臨床的研究を行なった．対象は各年令層にわたる急性気

道感染症，急性下痢症，発疹症その他で，ウイルス分離に主意を注ぎ，ペア血清の採取は136名にとどまった．ウイルス分離に供したのは咽頭ぬぐい液（あるいはうがい液）710，糞便（あるいは直腸ぬぐい液）461，眼結膜ぬぐい液117，計1,228検体で，主として急性期に採取したが一部回復期に得たものもあった．ウイルスの分離はHeLa細胞を用いた組織培養法によって行ない，3代継代80日間の培養中，細胞変性のないものを分離陰性とした．

アデノウイルスは470名の乳幼児・小児中48名より49株が分離され，1型3株，2型9株，3型23株，5型9株，6型1株，11型1株，未同定2株であった．なお，15才以上の成人206名からは本ウイルスは分離されなかった．これらの型別所見を年令別にみると，3型は主として学童から，2および5型は主に乳児から，他の型はほとんど幼児から検出された．疾患別には上気道感染症を伴う疾患群の患児からは11型を除くすべての型が分離されたが，乳児白色便性下痢症からは5，6，11型が，猩紅熱様，あるいは風疹様発疹を呈するものからは2，5型が分離された．検体別分離率は咽頭材料5.1%，糞便材料6.7%で，眼結膜ぬぐい液からはウイルスは分離されなかった．一方，咽頭材料と糞便材料共に採取できた350名からのウイルス分離を比較したが，両材料共に陽性のものが大部分を占めるなかで，糞便のみ陽性の若干の例がみられた．次に検体の採取病日による分離率を材料別に検討したが，アデノウイルス分離の最適病日は，咽頭材料では第4病日，糞便材料では第4病日から第21病日頃までであると考えられた．第1病日の採取検体からは糞便および咽頭材料共に全く本ウイルスは分離されず，この点留意すべき所見と思う．検体は採取後各施設で4°Cあるいは室温に保存されその後に研究所に運ばれたので，一部には分離実施に先立ち5日以上そのまま放置のものも含まれていた．これらの検体からのウイルス分離状況を放置の日数や温度について一応検討したが，分離率にはほとんど差がみられなかった．

血清補体結合反応でアデノウイルス感染症と診定し得たものは136名中25名(18.4%)であった．成人からのアデノウイルスの分離は既述のように陰性であったが，37名中5名がこれによって4倍以上の抗体上昇を示した．

アデノウイルス分離の過程で，29名より単独あるいはアデノウイルスと共存の形でエンテロウイルスが分離され，そのうち6株はポリオウイルス1，2，3型，コクサッキーウイルスB3型およびエコーウイルス6型と同定された．以上のほか，ウイルス分離によりあるいは血清学的に診定されたアデノウイルス感染症の臨床所見を要約し，特に発疹を呈した6例についてはこれを詳記した．