monella, Shigella and other *Enterobacteriaceae,* some of which have not been previously isolated from rabbits, and that this anamnestic rise frequently gives titers higher than those achieved with *E. coli* 014; 2) *E. coli* 014, and perhaps 056 and 0144, were the only antigens to induce such a phenomenon, while other equally active 0 antigens failed to do so; 3) inhibition and absorption studies indicate that any one of a large number of unrelated *Enterobacteriaceae* antigens can block the 014 antiserum cross reactivity, but only the 014 antigen can block its reaction with 014 serum; 014 antigen does not block combination of heterologous 0 antigens with their corresponding sera suggesting that the antibody produced is quite different than those classically associated with 0 antigens; 4) only the hemagglutination test is involved in the 014 phenomenon; no response of agglutinating or precipitating antibodies was observed as might be expected if the alternate hypothesis were correct or if an antigen other than a hapten were involved.

Summary. Sera from rabbits immunized at 3 different laboratories with *E. coli* 014 antigens prepared from boiled whole cultures or with Boivin type extracts agglutinate human and sheep erythrocytes coated with lipopolysaccharides or crude 0 antigen type preparations from a wide variety of *E. coli* and other *Enterobacteriaceae* but do not produce heterologous bacterial agglutination or precipitin formation. Inhibition and absorption studies suggest that the observed phenomena are due to a hapten associated, but not necessarily identical, with the crude endotoxin moiety of *Enterobacteriaceae* which is so structured in *E. coli* 014 as to be able to induce antibody formation.

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Observations on Some Little-Known Adenoviruses. (27735)

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Considerable information has been published on the natural history of certain adenoviruses, *viz.,* types 1, 2, 3, 4, 5, and 7(1). Relatively little data are available on the other serotypes. This report summarizes observations made on some of the little-known

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serotypes during a longitudinal study of the microbial experiences of children living in an institution (Junior Village) in Washington, D. C.(2).

Materials and methods. General objectives, over-all plan of the longitudinal study, and characteristics of the study population have been described(2), and clinical and laboratory methods employed have been reported (2,3,4). Where pertinent, specific details of isolation procedures will be described with the findings. All viruses were typed by the hemagglutination-inhibition (HI) technic. Many of these identifications were confirmed by neutralization tests.

Results. The little-known adenoviruses to be discussed here include types 9, 10, 12, 13, 16, 17, 24, 25, 26, 27, 28, BP-6, and BP-7. These viruses differ from adenovirus types 1, 2, 3, and 5, which are commonly isolated from children, in that a) they are isolated almost exclusively from anal specimens; b) they are usually less readily isolated from a given specimen than are types 1, 2, 3, and 5; and c) the cytopathic effect produced by most of the types is distinct from that produced by types 1, 2, 3, and 5.

Virus isolations. During the first 3 years of the longitudinal study (July, 1955 to June, 1958) anal specimens were tested *routinely* only in rhesus kidney cultures. Since the adenoviruses under consideration can only rarely be isolated in this tissue, and cannot be passed in it, they were encountered during this time-period primarily when *special* tests with tissue cultures of human origin were carried out.

From July, 1958 to June, 1959, anal as well as throat specimens were tested routinely in KB cultures. The initial cultures were kept for 7 days after inoculation without a fluid change and then passed routinely to fresh KB cultures. The latter were also kept 7 days without a fluid change, then routinely tested for the presence of adenovirus complement-fixing antigen.

The number of infections with selected adenovirus serotypes which were detected by virus isolation in the 4-year period from July, 1955 to June, 1959 is shown in Table I. There was a tendency for certain serotypes to predominate in particular time periods. For example, types 13 and 25 were encountered only in 1955 and 1956 even though testing was more adequate in later years. Type 10, however, was isolated in each of the 5 years and type 26 was encountered in 4 of the five. In addition to the serotypes listed in Table I and numerous infections with types 1, 2, 3, and 5, three infections with type 6 and 6 infections with incompletely classified adenoviruses were detected during the 4-year period.

TABLE I. Number of Infections with Selected Adenoviruses in Junior Village by Serotype and Year.

Serotype	1955	1956	1957	1958	1959
T 9			6	6	1
T10	3	1	4	11	5
T12				2	1
T13	2	5			
T16			4	61	4
T17			1	9	
T24				1	1
T25	1	4			
T26		5	3	29	2
T27			2	8	2
T28				5	
BP-6				1	3
BP-7					3

Data on the frequency with which adenoviruses of all types were isolated from throat and anal specimens during the one-year period beginning at the end of June, 1958 are shown in Table II. During this year all rou-

TABLE II. Adenoviruses Isolated from Paired Throat and Anal Specimens of Children in Junior Village by Date of Specimen Collection.

Date of specimen collection (1958-1959)	No. of pairs			
	Tested	With throat only positive	With anal only positive	With both throat and anal positive
6/26- 7/23	51	0	5	0
7/24- 8/20	47	1	1	0
8/21- 9/17	67	0	1	0
9/18-10/15	71	0	1	0
10/16-11/12	63	1	4	0
11/13-12/10	75	0	11	0
12/11- 1/ 7	58	0	3	0
1/ 8- 2/ 4	70	1	7	0
2/ 5- 3/ 4	66	0	3	1
3/ 5- 4/ 1	71	5	7	0
4/ 2- 4/29	95	5	9	2
4/30- 5/27	77	0	6	3
5/28- 6/24	125	4	12	2
Totals	936	17	70	8

tine throat and anal specimens collected every fourth week and all admission throat and anal specimens were tested in KB cultures as described above. Only those specimen pairs in which both the throat and anal specimens were successfully tested are included in the tabulation. The anal specimens yielded more adenovirus isolates than did the throat specimens. This higher rate prevailed throughout the year. When the isolations from the 2 types of specimens were tabulated by serotype, it was found that all isolates from throat specimens only, or from both throat and anal specimens, were types 1, 2, 3, and 5. The same serotype was recovered from the throat and anal specimen in the 8 instances where both specimens were positive. Of the 70 isolates obtained from anal specimens only, 36 were types 1, 2, 3, and 5; and 34 were types 10, 12, 16, 24, 26, 27 or incompletely classified.

Thus, of 95 specimen pairs found positive by routine testing during this year, 61, or 64%, yielded types 1, 2, 3, and 5 and the rest had other serotypes. If only throat specimens had been tested, 25 isolates (26% of the actual total) would have been obtained and all of these would have been types 1, 2, 3, and 5. Considering only the latter types, 44 of 61 (72%) would have been detected by testing anal specimens only and 25 of 61 (41%) by testing throat specimens only. Although only types 1, 2, 3, and 5 were detected in throat specimens during the one-year testing period summarized in Table II, a few isolates of types 9, 10, and 16 were obtained from throat specimens in previous years.

Examination of the limited data available from a number of miscellaneous special tests over the 4-year study period indicated that adenovirus types 1, 2, 3, and 5 were apparently excreted for as long a period in the throat as in the feces. Moreover, the time of first appearance of these serotypes in the throat did not appear to precede that of their appearance in the feces. Most of the isolates of serotypes other than types 1, 2, 3, and 5 were recovered from only a single specimen of a given child. Sometimes, however, these isolates were obtained from serial anal specimens covering a span of from one to 4 weeks. This duration of excretion did not appear to occur more frequently with these serotypes than with types 1, 2, 3, and 5.

TABLE III. Adenoviruses Isolated from Admission Specimens of Children in Junior Village by Serotype and Month.*

Month (7/56-6/59)	Types 1,2,3,5	Types 9,10,12,16
January	4	1
February	0	0
March	1	3
April	1	0
May	3	2
June	3	0
July	0	0
August	0	1
September	1	0
October	0	0
November	1	0
December	3	1
Totals	17	8

* Only viruses isolated from specimens obtained 2 days or less after admission are included — see text for further details.

The number of adenovirus isolates obtained from admission specimens of children entering Junior Village from July, 1956 to June, 1959 is shown in Table III. Only those specimens collected 2 days or less after admission are included. In those instances where both the throat and anal specimen were positive, only one of the isolates is included in the tabulation. It will be noted that there was no striking seasonal introduction of either types 1, 2, 3, and 5 or the other serotypes which were encountered in admission specimens.

Serology. Since the adenoviruses other than types 1, 2, 3, and 5 were usually isolated only with difficulty, and since testing for these viruses in Junior Village had been limited, an attempt was made to employ serologic methods to estimate their prevalence. Preliminary experiments carried out with the sera of children yielding isolates of types 9, 10, 13, 17, 26, 27, BP-6, and BP-7 indicated that a large proportion of individuals responded with a significant rise in homologous HI antibody titer. Specifically, of 60 children with isolates of types 9, 10, 26, or 27, and pre-isolation

homologous titers of less than 1:10, 44 (73%) showed rises to 1:20 or greater and 8 others (13%) showed rises to 1:10.

On the other hand, significant HI antibody responses were not detected in children known to have been infected with types 16, 24, 25, and 28. As only a few serum pairs were available from children infected with the latter 3 serotypes, the significance of findings for these types is difficult to evaluate. With type 16, however, extensive testing was carried out with negative results. Even the use of a prolonged serum-virus incubation period (overnight at room temperature) and homologous virus as antigen failed to demonstrate HI antibody with the latter type. When some of the type 16 serum pairs were tested for neutralizing antibody it was found that even this type of antibody could be detected only with difficulty, and only in some of the pairs.

A serologic survey for infection with types 9, 10, 26, and 27 was carried out among the children present in Junior Village from Jan. 1, 1957 to June 30, 1959. These serotypes were selected because they were known to have occurred in the population during this time interval and because they were known to give rise to homologous HI antibody responses in most infected children. Only children continuously present in the population for 4 weeks or longer were studied. The only other criterion for inclusion in the survey was availability of appropriate sera for testing. Serum pairs collected at least 4, and not more than 24, weeks apart were first screened by HI and, when indicated, titrated against each of the 4 viruses mentioned above. In most instances the initial serum of each pair was the first serum collected from the child after admission to the institution.

Considering first the results of testing the initial sera collected 14 days or less after admission, it was found that 32 of 254 (13%), 61 of 254 (24%), 37 of 254 (15%), and 18 of 254 (7%), had HI antibodies of titers of 1:10 or greater against adenovirus types 9, 10, 26, and 27 respectively. A total of 101 of 254 (40%) had HI antibodies to at least one of the above 4 serotypes. Approximately 500 children were admitted to Junior Village during the time period under consideration and hence sera from about one-half of the children admitted were tested.

TABLE IV. Number and Percentage of Children in Junior Village Who Developed HI Antibody Rises to Selected Adenovirus Serotypes by Time-Interval between Serum Specimens.

Time-interval between serum specimens (wk)	No. children tested	No. children with 4-fold rises	% children with 4-fold rises
	Type 9		
4- 5	28*	1	4
6-11	70*	14	20
12-23	85*	32	38
	Type 10		
4- 5	24*	6	25
6-11	62*	13	21
12-23	72*	28	39
	Type 26		
4- 5	25*	2	8
6-11	65*	13	20
12-23	85*	41	48
	Type 27		
4- 5	26*	2	8
6-11	82*	21	26
12-23	90*	44	49
	Type 9, 10, 26 or 27		
4- 5	19†	5	26
6-11	51†	25	49
12-23	68†	55	81

* Only children with initial titers of <1:10 to homologous virus are included.

† Only children with initial titers of <1:10 to all 4 types are included.

The number and percent of children with 4-fold HI antibody rises to each of the 4 serotypes is shown in Table IV by the time-interval between serum specimens. Only children with initial antibody titers of less than 1:10 against the homologous virus are included in this tabulation. A titer in the second serum of at least 1:20 was considered a 4-fold rise. Children with pre-existing homologous antibody developed 4-fold rises in only a few instances. An appreciable percentage of the children tested developed significant titers of HI antibodies against each of the test viruses. As would be expected, the percentage increased with the time-interval between serum specimens.

Examination of the serologic data for each of the serotypes with respect to time failed to reveal any striking grouping of antibody rises

to any of the viruses in particular time periods.

The specificity of the serologic data was studied in two ways. First, 39 children were selected for special virus isolation study. These were individuals a) who showed 4-fold rises from initial titers of less than 1:10 against one or more of the study serotypes, and b) who had not previously had an adenovirus isolated from any of their specimens in the time-interval bracketed by their paired sera. All available anal specimens from these children were tested in KB cultures by the procedure outlined above for the routine 1958-1959 specimens. In 14 instances (36%) an adenovirus of the serotype corresponding to the one type of antibody rise for which the child had been selected was isolated. The number of children with homologous isolates and the number tested for each of the serotypes was 3 of 9, 2 of 7, 5 of 11, and 4 of 12 for types 9, 10, 26, and 27 respectively. Other adenovirus serotypes were also isolated but no particular associations were noted.

Secondly, the data on paired sera with time intervals of from 6 to 11 weeks were analyzed to determine if multiple antibody rises to the 4 serotypes under consideration occurred with unusual frequency. It was found that 4-fold or greater responses to 2 or more viruses in a single serum pair were significantly more common than would be expected by chance alone. However, antibody rises to one virus did not appear to be linked to rises with any other single serotype.

Discussion. These data indicate that adenovirus serotypes other than types 1, 2, 3, and 5 were quite common among the children studied, and that the former viruses were recovered almost exclusively from anal specimens. Moreover, types 1, 2, 3, and 5 were more frequently isolated from routine anal specimens than from routine throat specimens in this population. In view of these findings and those of others(5,6,7) on the relationship of adenoviruses to the lower intestinal tract it would appear that reconsideration should be given to the common characterization of all adenoviruses as "respiratory viruses." While the latter term may be appropriate for a few of the adenovirus serotypes it is not for most of the viruses considered in this report.

While the adenovirus serotypes other than types 1, 2, 3, 5, and 6 recovered in Junior Village had in common the characteristic of being found almost exclusively in anal specimens, they were by no means an entirely homogeneous group. Some of the serotypes fall in hemagglutinating group 1 (types 16, 25, and 28) and others in group 2 (types 9, 10, 13, 17, 24, 26, 27, BP-6, and BP-7) or in none of the groups (type 12)(3). Even within these groups there are differences such as the radically different type of cytopathic effect in rhesus kidney cultures produced by type 16 as compared to types 25 and 28.

While the little-known adenoviruses discussed here were found in anal specimens, their epidemiology would appear to be different from that of the enteroviruses encountered in the same type of specimen in the same population. Most of the latter viruses appear to be disseminated much more rapidly in this institution, and as a result persist for much shorter periods of time. Another indication of a difference in epidemiology is the finding that the adenoviruses were not more commonly introduced into the Junior Village population in the summer and fall as were the enteroviruses. Although enteroviruses spread within the institution quite readily at any time of the year it has been shown that they were introduced most frequently in the summer and fall months(8).

Although it appears that children infected with most of the little-known adenovirus serotypes develop homotypic HI antibodies, the specificity of these responses has not yet been adequately explored. Adult human volunteers experimentally infected with adenovirus type 26 have shown some heterotypic HI responses (9) as have adults naturally infected with types 4 and 7(10). It would not be surprising, therefore, to find that some of the HI antibody rises detected in this study were of heterotypic origin. Because of the simultaneous presence in the institution of all 4 serotypes studied, it was not possible to determine what the proportion of heterotypic responses might be.

Although no homologous HI antibody re-

sponses could be detected in children yielding type 16 isolates, there were abundant data to indicate that the children themselves were the source of these viruses and not the tissue culture system. For example, in the routine 1958-59 testing type 16 isolates were obtained only from anal specimens despite the fact that both throat and anal specimens were tested simultaneously in the same tissue cultures.

Because of the frequency with which undifferentiated illnesses occur in the Junior Village population and the infrequent testing for the little-known adenoviruses, no information was obtained on the relationship of these agents to the common illnesses in the population.

Summary. The occurrence of a number of little-known adenovirus serotypes in children in an institution in Washington, D. C., is described. These viruses, which included types 9, 10, 12, 13, 16, 17, 24, 25, 26, 27, 28, BP-6, and BP-7 were isolated almost exclusively from anal specimens. As a group they were almost as common as the better known serotypes 1, 2, 3, and 5. It was shown that the latter types, although frequently present in routine throat specimens, were also more frequently isolated from routine anal specimens. Homotypic hemagglutination-inhibition antibody rises were demonstrated in children infected with most of the little-known serotypes.

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Source and Time of Release of Histamine-Destroying Factor (Histaminase) During Anaphylactic Shock in Rats.* (27736)

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Code and associates(1) have recently demonstrated the release of a histamine-destroying factor during anaphylactic shock in albino rats. This factor was released during shock whether the rats were sensitized using pertussis vaccine as an adjuvant or were adrenalectomized prior to sensitization without an adjuvant. The amount released in adrenalectomized rats was less than in intact rats. The factor has been tentatively identified as histaminase.

Waton(2) has shown that most of the histaminase in normal rats is found in the duodenum, ileum, and colon. Some of the enzyme, however, may be present in the stomach, liver, kidney, and lung. Lindell and Westling(3) have demonstrated that in the guinea pig the kidney and intestinal tract contain only small amounts of histaminase whereas the liver is rich in the enzyme. Our study was undertaken to determine the source of the histaminase released into the blood stream of the rat during anaphylaxis.

Method. White rats, mostly males of the Sprague-Dawley strain, weighing 150 to 260 g were used. The intact animals were sensitized by a single intraperitoneal injection of

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