pared with uptake from synthetic inorganic solutions and emphasize the difficulty of removing Sr^{85} from bone with chelating agents now available.

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Four Newly Recognized Adenoviruses. (26648)

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During a longitudinal study of the microbial experiences of children in an institution in Washington, D. C.(1) more than 50 strains of adenoviruses were isolated which could not be identified as any of the known types(2,3). Further study revealed that these viruses could be grouped into the 4 previously unrecognized serotypes which are described in this report.

Materials and methods. Prototype adenovirus strains were obtained from Drs. W. P. Rowe and S. D. Bell, Jr. The "S-1058" strain of type 7A was used as the representative of type 7. Neutralization tests were carried out in Rhesus kidney cell cultures(2) employing "procedure 2" of Rowe, *et al.*(4). Hemagglutination and hemagglutination-inhibition (HI) tests were performed as described previously(5). Rabbit antisera were used in both neutralization and HI tests(5). Complement-fixation tests were done by a standard technic which has been used with a number of viral antigens(4).

Throat and anal swabs were collected, stored, and inoculated by the methods outlined previously(6). Isolates were recovered in a variety of tissue cultures of human origin maintained for varying periods of time on several different kinds of media. At least one strain of each type, however, was isolated in either HeLa or KB cells. The latter tissues had been grown on a medium consisting of Eagle's basal medium with 10% inactivated human serum and were maintained on medium No. 199 with 5% inactivated chicken serum. After inoculation, KB and HeLa cultures were observed for cytopathic effects (CPE) for 7 to 12 days, when at least one blind passage was made. Maintenance fluid was usually changed every 2 or 3 days, but in some instances changes were omitted in cultures observed for only 7 days.

Results. The viruses described here appeared to be less readily isolated from a given specimen in this study than did adenovirus types 1, 2, 3, and 5. In general, the CPE of the new serotypes was slower to appear than that of the latter viruses and in

many cases a definite CPE was not seen until one or 2 blind passages had been made. The nature of the CPE of the new serotypes in unstained cultures of both Rhesus kidney and KB cells was also distinctly different from that of types 1, 2, 3, and 5, but was indistinguishable from that of types 9, 10, and 13. As was the usual experience with types 9, 10, and 13 in this study, and again in contrast to the findings with types 1, 2, 3, and 5, isolates of the new viruses were obtained only from anal specimens. Throat and anal specimens taken from the same child at the same time were almost always tested in parallel.

Some of the children yielding isolates of the new serotypes had minor illnesses of a variety of types. However, minor illnesses were extremely prevalent in this particular study population and the data available were not sufficient to determine if the newly recognized adenoviruses were etiologically associated with any of them.

After all untyped adenovirus isolates had been grouped into 4 serotypes by means of HI tests, one strain of each type was selected as the prototype and all further studies were carried out with these strains unless otherwise noted. The origin of the 4 prototype strains was as follows.

The strain selected as the prototype of the virus which will be referred to as BP-1 was isolated from an anal specimen collected from a 16-months-old female Negro child on Aug. 1, 1956. Four other strains of this type were isolated from the same study population, one in 1955 and the others in 1956.

The strain selected as the prototype of the virus which will be referred to as BP-2 was isolated from an anal specimen collected from a 9-months-old male Negro child on July 16, 1956. Many other strains were isolated from the same population in 1956, 1957, 1958, and 1959 as well as from children in Saudi Arabia(7). This serotype has been referred to previously as BAR-2(8).

The strain selected as the prototype of the virus which will be referred to as BP-4 was isolated from an anal specimen collected from a 9-months-old female Negro child on Feb. 24, 1958. Five other strains of this type were isolated from the same population, one in 1957 and the others in 1958.

TABLE I. Homologous Hemagglutination-Inhibition Titers of Prototype Antisera.

Virus type	Homologous titer	Virus type	Homologous titer
1	320*	15	640
2	320	16	320
3	5120	17	640
4	320	18	†
5	320	19	80
6	320	20	80
7	2560	21	160
8	1280	22	1280
9	2560	23	2560
10	640	24	160
11	80	BP-1	80
12	†	BP-2	5120
13	1280	BP-4	320
14	320	BP-5	80

* Reciprocal of serum dilution.
† Homologous HI titer not obtainable because virus does not hemagglutinate. Neutralization titers of types 12 and 18 sera were $\geq 1:800$ and 1:80 respectively.

The strain selected as the prototype of the virus which will be referred to as BP-5 was isolated from an anal specimen collected from a 30-months-old male Negro child on April 23, 1958. Four other strains of this type were isolated from the same population, all in 1958.

With the exception of BP-1 virus, a rise in neutralizing antibody was demonstrated with each of the new types in one or more of the children yielding isolates. Only a single pair of sera from a child with a BP-1 virus isolate was available and no neutralizing antibody was detected in either serum. Two strains of BP-1 virus were reisolated, however, from the original specimens indicating that the viruses had originated there and not in the tissue culture system.

A sample of each of the new serotypes was found to have the same titer in Rhesus kidney cell cultures after treatment with 20% ethyl ether for 18 hours at 4°C as did a control sample similarly treated with 0.85% NaCl.

Using each of the new serotypes as an antigen, a significant rise in complement-fixing antibody titer was demonstrated in paired sera from three individuals each of whom was known to have been infected with a different previously described adenovirus.

None of the new serotypes could be passed serially in Rhesus kidney cultures and their CPE as seen in unstained Rhesus kidney cultures in primary passage was distinctly different from that of the chimpanzee and monkey adenoviruses described by Rowe *et al.* (2).

Each of the new prototype viruses was set up in neutralization tests against antisera of each of the other human serotypes. The homologous titers of these sera ranged from 1:80 to 1:320 or greater and they were used initially at a dilution of 1:5 or 1:20. Conversely, antisera for each of the new types was tested against each of the other viruses. The titer of the BP-1 serum was 1:80 and that of the BP-2, BP-4, and BP-5 sera was 1:320 or greater. The BP-1 serum was used initially at a dilution of 1:5 and the others at a dilution of 1:20. The heterologous neutralizations observed were as follows. BP-2 antiserum had a titer of greater than 1:20 but less than 1:80 against both type 9 and BP-4 virus. BP-1 antiserum had a titer of greater than 1:20 but less than 1:80 against type 10 virus and a titer of 1:80 against type 15 virus. The antiserum for type 15 virus, which had a homologous titer of 1:320, had a titer of greater than 1:20 but less than 1:80 against BP-1 virus.

Each of the new viruses was also set up in HI tests against antisera of each of the other human adenovirus serotypes. Conversely, antisera of each of the new types were tested against each of the other viruses with the exception of types 12 and 18 (which do not hemagglutinate). Types 20, 21, BP-1, and BP-5 agglutinate Rhesus but not rat erythrocytes, and types 19, 22, 23, 24, BP-2, and BP-4 agglutinate rat cells completely. The homologous HI titers of the sera used are shown in Table I. No heterologous inhibition was observed between the 4 new types, or between these types and the 24 previously described serotypes, when the sera were used at a dilution of 1:10.

Discussion. The data presented indicate that the 4 virus serotypes described belong in the adenovirus family and that they were derived from human beings. With the exception of the BP-1 virus, each appears to be clearly distinct from the 24 previously described human adenoviruses by both neutralization and HI tests.

Although the BP-1 serum had the same neutralizing titer against type 15 virus as it did against its homologous virus, it is proposed that BP-1 virus be considered a distinct serotype, rather than a "prime" strain of type 15, for the following reasons. Firstly, BP-1 virus does not agglutinate rat erythrocytes whereas type 15 virus agglutinates these cells to high titer. Secondly, no crossing was observed between BP-1 and type 15 in reciprocal HI tests. Types 7 and 7A(2) are indistinguishable in reciprocal HI tests (5). Finally, certain problems have been encountered with adenovirus neutralization tests which suggest that data obtained by this technic should be considered with some caution. For example, adenovirus isolates have been encountered which were *doubly* neutralized by antisera of types 3 and 7, 14 and 16, and 9 and 15 respectively. In each instance the isolates had the hemagglutinating characteristics of only one virus of the pair and were inhibited only by the serum of that same virus(5). In the publication (2) which describes adenovirus type 15 it was noted that the antiserum for this virus had a titer against adenovirus type 4 only 4-fold lower than its homologous titer. Type 4 virus is clearly different from type 15 in CPE, hemagglutinating properties, and other characteristics.

After reviewing the data presented here, the Adenovirus Committee of the Nat. Inst. of Allergy and Infectious Diseases recommended that BP-1, BP-2, BP-4, and BP-5 viruses be designated adenovirus types 25, 26, 27, and 28 respectively.

Summary. A number of strains of adenoviruses recovered from anal swabs of children in Washington, D. C., were found to belong to the 4 previously unrecognized adenovirus serotypes (BP-1, BP-2, BP-4, BP-5) described in this report. The Adenovirus Committee of the Nat. Inst. of Allergy and Infectious Diseases has recommended that these viruses be designated adenovirus types 25, 26, 27, and 28 respectively.

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Effect of a Necrogenic Yeast Diet on Viral Hepatitis (M.H.V.$_3$) in Mice.* (26649)

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Certain yeast diets have long been known to produce liver necrosis in rats(1). In mice DeWitt and Schwarz(2) have recently produced necrosis affecting the liver, heart and some other organs by using a diet in which dried Torula yeast was the sole source of protein. They also showed that these lesions can be prevented by supplementation with inorganic selenium or alpha tocopherol. In chicks a Torula yeast diet produces exudates in muscle, fat and skin which can be prevented by the same supplements(3). We have investigated the effect of the necrogenic diet on experimental hepatitis produced in mice by M.H.V.$_3$ virus(4). It had previously been shown that an increase in protein intake greatly reduced the mortality from this infection. This effect could not be reproduced by individual amino acids(5) or selenium.‡

Methods. Female weanling Swiss Webster mice were used. Repeated blood examinations had shown these mice to be free of Eperythrozoon coccoides, a protozoan which enhances the severity of the hepatitis. The principal constituents of the experimental diets are shown in Table I. The necrogenic diet (Diet I) was similar to that of DeWitt and Schwarz(2). Diets II and III were identical with Diet I except for supplementation with selenium and alpha tocopherol respectively. Diet IV was similar to Diet I except for substitution of casein for yeast. Since only about half the yeast consists of protein, the 15% of casein in Diet IV supplied approximately the same amount of protein as the 30% of yeast in Diet I.

Groups of mice aged 17 days were fed unrestricted quantities of the various diets. Unlimited fresh water was supplied. The animals were weighed twice weekly. After approximately 4 weeks a few deaths started to occur in Group I, the members of which were fed the necrogenic yeast diet. After 31 days half the animals of Group I and most of those on Diets II, III and IV were inoculated intraperitoneally with 0.1 ml of the M.H.V.$_3$ virus preparation(7). This consisted of a 10% suspension in Gey's balanced salt solution of mouse liver recently obtained from infected animals and stored at −30°C. Cages were inspected daily and dead mice removed. Some in each group were autopsied.

Results. In the first experiment mice fed the necrogenic diet (Diet I) were compared with animals receiving a diet identical apart from supplementation with alpha tocopherol (Diet II). Both groups were compared with

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