

Neutralizing antibodies against 33 human adenoviruses in normal children in Rome

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SUMMARY

There are few data about the distribution of neutralizing antibodies (NA) against adenovirus types in the Italian population, especially the high-numbered ones. We tested the sera from 453 children and 51 young adults to evaluate NA against adenovirus prototypes 1–33. Using the microneutralization test, 338 (74·6%) of the children's sera were positive for at least one adenovirus type. Antibody to type 2 was the most frequently detected followed, in descending order, by antibody to types 5, 1 and 3. All these types are known to be associated with disease but antibody to type 7, a type also associated with disease, was less frequent than that to other serotypes such as 18 and 31, the pathogenicity of which in man is not clearly established. The antibody positivity rate rose with age for the more frequent types while it did not vary for the less frequent ones. The number of sera with NA against more than one adenovirus type increased with age. With regard to types 1–8, we found that their frequencies in Italy were similar to those found in the U.S.A.

INTRODUCTION

The importance of some adenovirus types in human disease is well known, while the role of other serotypes is more obscure, especially that of the high numbered ones. Only 12 out of 36 adenovirus types (33 recognized, 2 candidates and 1 proposed recently (Wigand, Gelderblom & Wadell, 1980)) have been related to disease, mainly respiratory and ocular. These are the first eight types and types 11, 14, 19 and 21 (Kasel, 1979).

Data on the observed frequencies of these types have shown that some of them are very common among different populations and seem to infect children early in life, while others infect them later and are less frequent (Jackson & Muldoon, 1975). Many studies have been done to establish the frequencies of isolation and their role in causing disease. It has also been shown that as many as one half of adenovirus infections are asymptomatic (Bell, Rowe & Rosen, 1962; Fox *et al.* 1969; Brandt *et al.* 1969). Information is still limited on the spread of nonpathogenic serotypes, since the data gathered hitherto came more from in patients than from the normal population, as a whole.

To understand the circulation of different adenovirus types in Italy, we have studied the distribution of neutralizing antibodies (NA) in normal subjects against all adenovirus serotypes. Our study included measurement of antibodies in the first years of life to assess the period during which these children may be more susceptible to an adenovirus infection.

MATERIALS AND METHODS

Sera

We tested 453 sera from children aged from 0 to 12 years and 51 sera from young adults aged from 19 to 40 years with a mean age of 23. All the subjects were living in or near Rome and were from similar socio-economical backgrounds. The children were subjects without any acute disease, coming to the hospital for examination prior to vaccination procedures or for surgical treatment. The adults were all healthy volunteers. All the sera were collected in the period from 1977 to 1979 and were stored at -20°C . At the time of their use, the sera were diluted 1:10 in Minimal Essential Medium (MEM) and were inactivated for 20 min at 60°C .

Virus

Adenovirus prototypes 1–33 were supplied by the American Type Culture Collection (ATCC). The strains were passaged 2–3 times in HEp-2 cells. Viral cell-free suspensions were obtained by three repeated freeze-thawings and one centrifugation at 1200 *g*. They were titrated by the 50% end-point method in microplates and were diluted to contain 100 TCID₅₀ in 0.025 ml. They were kept at -40°C until the time of their use.

Procedure

Both virus titration and neutralization tests were performed in plates with 96 flat-bottomed wells (IS-FB 96 TC Linbro Scientific Inc., Hamden, Connecticut). A single plate was used to test 90 different sera against one serotype. Each well received 0.025 ml of serum and 0.025 ml of viral suspension. Two wells were used for cell control receiving 0.05 ml of MEM. The last four wells received 0.025 ml of MEM. They were used to check the virus titre by inoculating the viral suspension and three 10-fold dilutions previously prepared from the same viral suspension. The plates were held at 37°C for 1 h in a 5% CO₂ incubator. Afterwards, 0.05 ml of a HEp-2 cell suspension were added to each well. The suspension contained 3×10^5 cells per ml of MEM containing 10% fetal calf serum. The plates, sealed with adhesive tape, were incubated at 37°C until the control CPE was ready to read, within 2–11 days depending on the serotype. Sera were considered positive if they neutralized the CPE completely when complete CPE was seen in the control well containing 100 TCID₅₀ as confirmed by the back titration.

RESULTS

By these neutralization tests we found 338 (74.6%) of the children's sera were positive for at least one adenovirus type, while the other 115 (25.4%) were completely negative. Only one of the 51 sera from young adults was negative.

Table 1. *Distribution of positive sera for at least one adenovirus type by age*

Age	Sera	
	Total	Positive (%)
< 6 months	74	38 (51·3)
6-12 months	42	14 (33·3)
12 months to 3 years	96	60 (62·5)
3-6 years	100	92 (92·0)
6-9 years	76	72 (94·7)
9-12 years	65	62 (95·4)
Adults	51	50 (98·0)

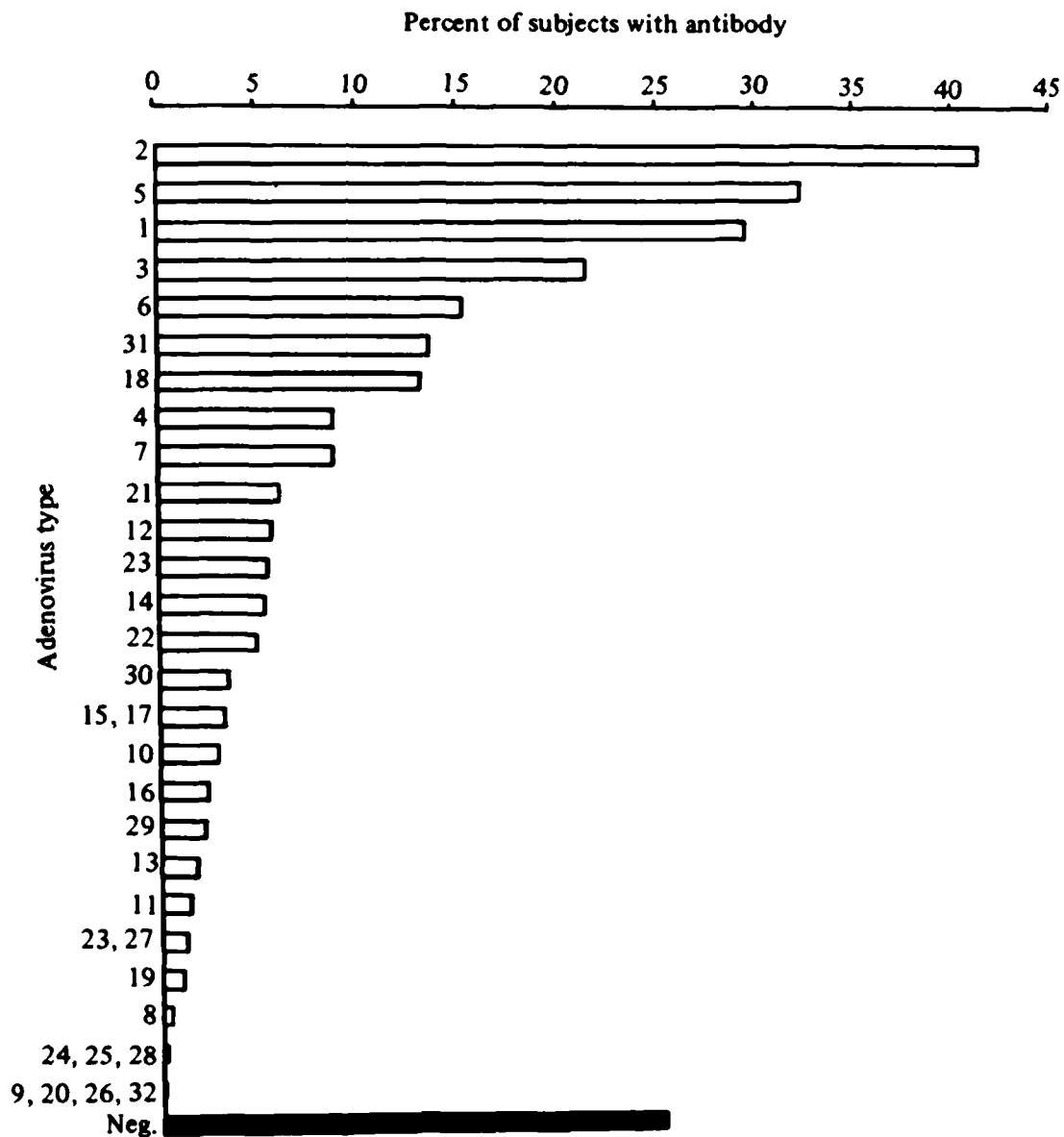


Fig. 1. Frequency of adenovirus antibody in children by type. The percentage of completely negative sera is shown at the bottom.

Table 1 shows the distribution of positive sera by age. It is noteworthy that 92% of children in the 3- to 6-year age group were positive for at least one serotype. The percentages of children's sera with antibody against each serotype are shown in Fig. 1. The highest percentage (41·5%) had antibody to type 2 and this was followed, in decreasing order, by types 5, 1, 3, 6, 31 and 18. Antibody to other

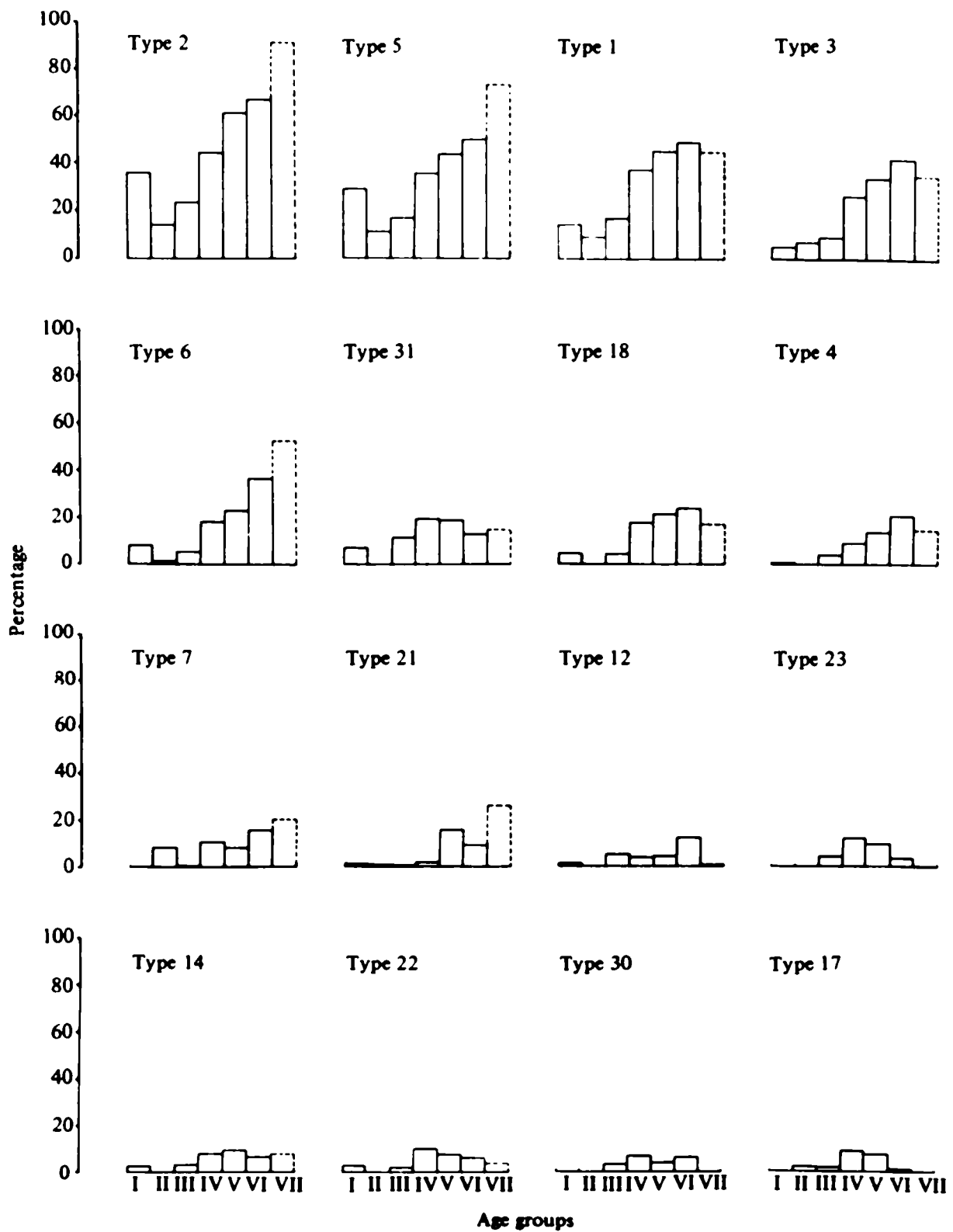


Fig. 2. Distribution of antibody against some adenovirus types by age. Sixteen serotypes in decreasing order of frequency of antibody are reported. The subjects were grouped as follows: I, < 6 months (74 subjects); II, 6-12 months (42); III, 12 months to 3 years (96); IV, 3-6 years (100); V, 6-9 years (76); VI 9-12 years (65); VII, young adults (51).

Table 2. Relationship between the number of types for which antibody was present and age groups*

Age	Total number of types neutralized						
	0	1	2	3	4	5	≥ 6
< 6 months	48.7	17.6	16.2	8.1	5.3	2.7	1.4
6-12 months	66.7	14.3	14.3	2.4	2.4	0	0
12 months to 3 years	37.5	20.8	18.8	11.5	7.3	3.1	1.0
3-6 years	8.0	21.0	20.0	20.0	14.0	7.0	10.0
6-9 years	5.3	10.5	27.6	18.4	10.5	9.2	22.4
9-12 years	4.6	7.7	12.3	15.4	21.5	12.3	26.2

* The numbers represent the percentages of children with antibodies against the indicated number of serotypes.

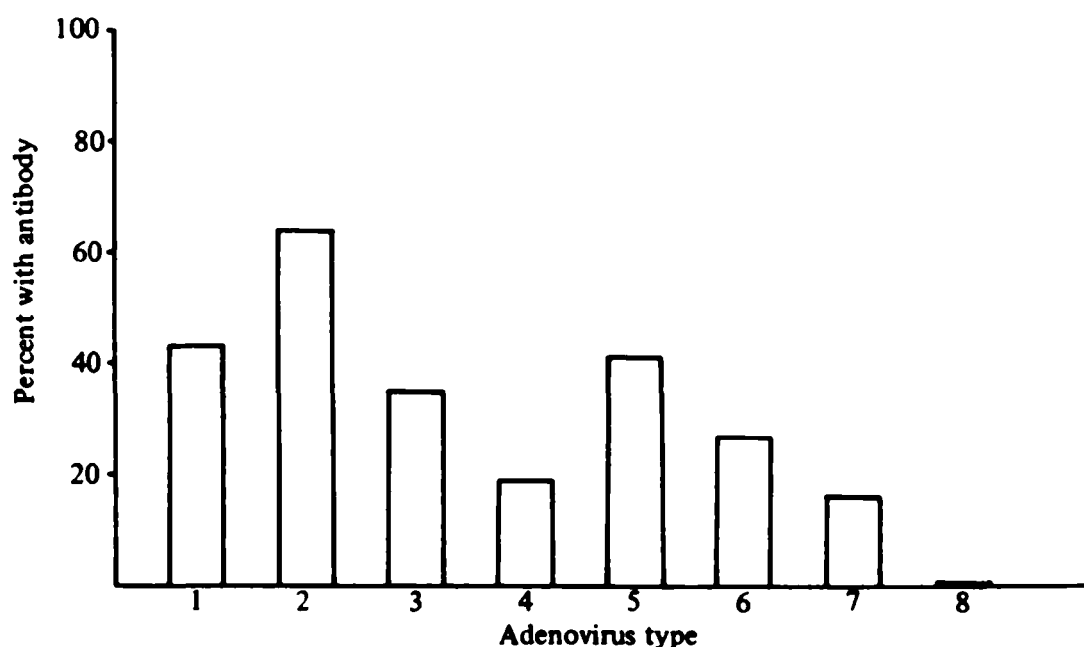


Fig. 3. Frequency of adenovirus antibody by type (1-8) in 149 primary school aged children (5-11 years old).

serotypes was present in less than 10% of the sera. Antibodies against types 9, 20, 26 and 32 were never found. Only two children had antibodies against type 8. Fig. 2 shows the distribution by age of antibody to some of the commoner serotypes. The percentage of positivity for the more frequent types rose steadily from the group 6-12 months to the group 9-12 years. Apparently, the percentage of positive sera for types 2, 5, 6, 7 and 21 is higher in adults than in children. The same result, not shown in the figure, was obtained for types 13 and 11. Except for types 3 and 7, all types showed a lower frequency in the second age group (6-12 months) than in the first (< 6 months).

The relationship between the number of different serotypes neutralized by the children's sera and their age is reported in Table 2. The percentage of subjects with antibodies neutralizing an increasing number of types rose with the subjects' age. About a quarter of children older than 6 years had antibodies against six or more

serotypes. The 6–12 months age group had the lowest percentage of children with antibodies against several types.

We selected the 149 primary school-children (5–11 years old) and showed the percentages of positive sera to adenovirus types 1–8 in Fig. 3.

DISCUSSION

Our study confirmed that adenovirus infections are contracted early in life (Potter & Shedden, 1963). At 12 months to 3 years of age, two-thirds of the children showed antibody to adenoviruses while this proportion rose to 92% in 6-year-old subjects. A very similar percentage was found in adults.

In general, epidemiological studies on adenovirus have only investigated the first eight numbered types which, with type 21, are those most frequently associated with disease. Our study, of all 33 accepted serotypes, also showed high frequencies of antibody to types 1, 2, 3 and 5. On the other hand, antibodies to types 4 and 7 were less frequent than to types 18 and 31, the frequencies of which were similar to that of type 6. Antibody to type 8, often associated with keratoconjunctivitis was very infrequent in our population. The distribution of antibody by age showed, for almost all types, a positive rate decreasing from the < 6-month age group to the 6- to 12-month age group that can be attributed to loss of maternal antibodies. Later in childhood, the positivity rate rose with age for the most frequent types, while it was steady for the less frequent ones. Since the percentage of positive subjects was not higher for all types in the adult group, a change in the epidemiology of these types in the last years can be suspected although the number of sera studied was small. In fact, if we consider only adult sera, the frequencies, in decreasing order, are the following: 2, 5, 6, 1, 3, 21, 7.

As previously reported (Potter & Shedden, 1963; Schmidt, Lennette & King, 1966; Taylor, 1977), the number of sera with antibodies against more than one serotype increased with age. Up to 3 years of age, most sera indicated infection with not more than two types while 60% of the 12-year-old subjects presented antibodies against four or more serotypes. Subjects with antibodies against two or three different adenoviruses had sera positive for the most frequent types, that is, they have different combinations of antibodies to types 2, 5, 1 and 3. However, antibodies against unusual types were also present in sera of subjects who had undergone many adenovirus infections. Since a constant coupling of two or more serotypes was never found, it would seem that the antibodies observed were really homotypic.

On the whole, our study confirmed that the low numbered types, except for type 8, are the most frequent adenoviruses in our country, but we also recorded a relatively high frequency of types 18 and 31. Although the distribution of adenovirus infections is said to be similar all over the world (Taylor, 1977), differences were noted in various countries (Foy & Grayston, 1976). The comparison of the frequencies of types 1–8 recorded by us with those reported in Japan, Taiwan, the U.S.A. (Tai & Grayston, 1962) and Sweden (Sterner, 1962) showed that the frequencies were similar in our country and in the U.S.A. while they were

different from those reported in oriental countries. These differences in countries, as well as the possible variation of epidemiological patterns from year to year and the recognition of new serotypes make adenovirus epidemiology unpredictable. Hence there is a need for repeating surveys from time to time.

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REFERENCES

- BELL, J. A., ROWE, W. P. & ROSEN, L. (1962). II. Adenoviruses *American Journal of Public Health* **52**, 902–907.
- BRANDT, C. D., KIM, H. W., VARGOSKO, A. J., JEFFRIES, B. C., ARROBIO, J. O., RINDGE, B., PARROTT, R. H. & CHANOCK, R. M. (1969). Infections in 18,000 infants and children in a controlled study of respiratory tract disease. I. Adenovirus pathogenicity in relation to serologic type and illness syndrome. *American Journal of Epidemiology* **90**, 484–500.
- FOX, J. P., BRANDT, C. D., WASSERMANN, F. E., HALL, C. E., SPIGLAND, I., KOGON, A. & ELVEBACK, L. R. (1969). The virus watch program: a continuing surveillance of viral infections in metropolitan New York families. *American Journal of Epidemiology* **89**, 25–50.
- FOY, H. M. & GRAYSTON, J. T. (1976). Adenoviruses. In *Viral Infections in Humans* (ed. A. S. Evans), New York: Wiley.
- JACKSON, G. G. & MULDOON, R. L. (1975). *Viruses Causing Common Respiratory Infections in Man*, p. 183. Chicago, London: University of Chicago Press.
- KASEL, J. A. (1979). Adenoviruses. In *Diagnostic Procedures for Viral, Rickettsial and Chlamydial infections*, 5th ed. (ed. E. H. Lennette and N. J. Schmidt). Washington, D.C.: American Public Health Association.
- POTTER, C. W. & SHEDDEN, W. I. H. (1963). The distribution of adenovirus antibodies in normal children. *Journal of Hygiene* **61**, 155–160.
- SCHMIDT, N. J., LENNETTE, E. H. & KING, C. J. (1966). Neutralizing, hemagglutination-inhibition and group complement-fixing antibody responses in human adenovirus infections. *Journal of Immunology* **97**, 64–74.
- STERNER, G. (1962). Adenovirus infection in childhood: an epidemiological and clinical survey among Swedish children. *Acta Paediatrica Scandinavica. Supplementum* **142**, 1–30.
- TAI, F. H. & GRAYSTON, J. T. (1962). Adenovirus neutralizing antibodies in persons on Taiwan. *Proceedings of the Society for Experimental Biology and Medicine* **109**, 881–884.
- TAYLOR, P. E. (1977). Adenoviruses: diagnosis of infections. In *Comparative Diagnosis of Viral Diseases* (ed. E. Kurstak and C. Kurstak). New York: Academic Press.
- WIGAND, R., GELDERBLUM, H. & WADELL, G. (1980). New human adenovirus (candidate adenovirus 36), a novel member of subgroup D. *Archives of Virology* **64**, 225–233.