J. Hyg., Camb. (1963), **61**, 155
Printed in Great Britain

The distribution of adenovirus antibodies in normal children

By C. W. POTTER and W. I. H. SHEDDEN
From the Department of Bacteriology, Sheffield University and the Children's Hospital, Sheffield

(*Received* 24 *October* 1962)

INTRODUCTION

Population surveys for the presence of adenovirus complement-fixing (C.F.) and neutralizing antibodies suggest that certain serotypes, particularly types 1 and 2, are endemic. Huebner, Rowe, Ward, Parrott & Betts (1954) estimated that, of children in Washington in the age group 6–11 months, over 50% had antibodies against one or more serotypes and 30% had antibody against type 1 or type 2. The percentages with neutralizing antibody against one or more viruses rose with age. Jordan, Badger & Dingle (1958) carried out a survey of 81 children in Cleveland over the first 5 years of life. This confirmed the endemic nature of adenovirus infection.

Whether or not a similar state of affairs exists in children of Great Britain, has never been determined. A study, similar to that of Huebner *et al.* (1954), is given here from Sheffield.

MATERIALS AND METHODS

Sera

Forty-two paired serum specimens of maternal and cord blood were supplied by Dr Bowley of the Sheffield Regional Blood Transfusion Centre, and in addition, 197 single serum specimens were collected over a 2-year period (November 1959 to November 1961) from Sheffield children coming to the Children's Hospital for examination prior to adoption or for treatment of burns, cleft palates and accidents. Specimens were not taken from children who had been previously in hospital or who had received previous blood transfusions or who were suffering from renal disease.

All serum specimens were stored at $-20°$ C.

Viruses

Adenovirus serotypes 1–7 inclusive were obtained from Dr M. H. Hambling of the Central Public Health Laboratory, Colindale. Viruses were grown on HEp 2 tissue cultures in 4 oz. medical flat bottles. Tissue cultures were harvested, when full cytopathic effect (C.P.E.) was observed, by three cycles of freezing and thawing. The cell debris was removed by centrifugation at 3000 rpm for 20 min. and the virus suspensions collected and stored at $-20°$ C. Infectivity titrations were carried out on HEp 2 tissue cultures in $4 \times \frac{1}{2}$ in. test tubes using logarithmic virus dilutions. Infectivity titres were calculated using the method of Reed & Muench (1938) on a three-day titration.

Control antisera

Rabbit hyperimmune adenovirus antisera were prepared by inoculating 5·0 ml. of neat virus suspension intravenously into rabbits on alternate days for 10 days. Test serum specimens were taken 3 weeks after the first inoculation and neutralization titres against homologous and heterologous adenovirus serotypes calculated.

Neutralization tests

Doubling dilutions of each serum were tested. Virus–serum mixtures containing 0·1 ml. of serum dilution and 0·1 ml. of virus containing 10 CPD_{50} (Binn & Hilleman, 1960) were prepared. These were left at room temperature for 1 hr. and then 0·3 ml. of maintenance medium (2 % inactivated calf serum in Medium '199') was added. The total volume, 0·5 ml., was then transferred to a tube of semi-confluent HEp 2 tissue culture cells previously washed twice with phosphate-buffered saline (Dulbecco & Vogt, 1954). For each serum dilution, two or more tissue culture tubes were inoculated.

Tubes were incubated at rest at 37° C. Virus controls showed specific C.P.E. after 24 hr. incubation and complete degeneration after 3 days. Serum neutralization titres were read after 3 days incubation and are given as the highest serum dilution showing 50 % or less C.P.E.

Complement-fixation tests

Complement-fixation tests were performed by the method of Pereira (1956).

RESULTS

Neonatal passive immunity

Forty-two pairs of sera from maternal and cord blood were tested for neutralization titres against adenovirus type 1 and 3. Serum dilutions from 1/10 to 1/320 in doubling dilutions were used. Titration end-points are given as the highest serum dilution showing 50 % or less C.P.E. when challenged by standard virus dose in HEp 2 tissue cultures.

Neutralizing antibody for adenovirus type 1 was found at 1/10 dilution or higher in 22 (52·4 %) of maternal serum specimens and in 25 (59·5 %) of corresponding cord blood specimens. Antibody was found in three foetal serum specimens at 1/10, but not in the corresponding maternal specimens. The average maternal antibody titre was 1/40, and the average cord titre was 1/50. The relative foetal/maternal antibody concentration was therefore 1·25/1·0.

Neutralizing antibody for adenovirus type 3 was detected in 19 (45·2 %) of both maternal and cord specimens. In one pair antibody was detected at 1/10 dilution of maternal serum, but not in the corresponding cord serum, and in one other case the converse was true. The average maternal titre was 1/100 and the average cord titre 1/115. The relative foetal/maternal antibody concentration was thus 1·15/1·0.

Disappearance of neonatal passively acquired adenovirus antibodies

Fifty serum specimens from children 1 year old or younger were tested for the presence of neutralizing antibody against adenovirus types 1, 2, 3, 5, 6 and 7. Figure 1 shows the number of serum specimens giving adenovirus neutralization

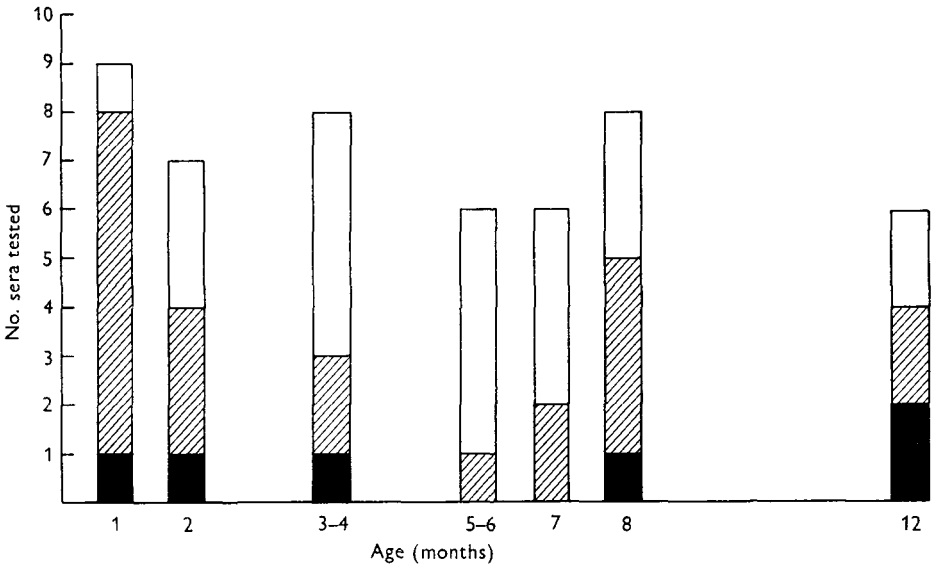

Fig. 1. Correlation of age with the presence of adenovirus neutralizing antibody. □, Number tested; ▨, number with neutralizing antibody; ■, number with antibody titres ⩾ 1 : 80.

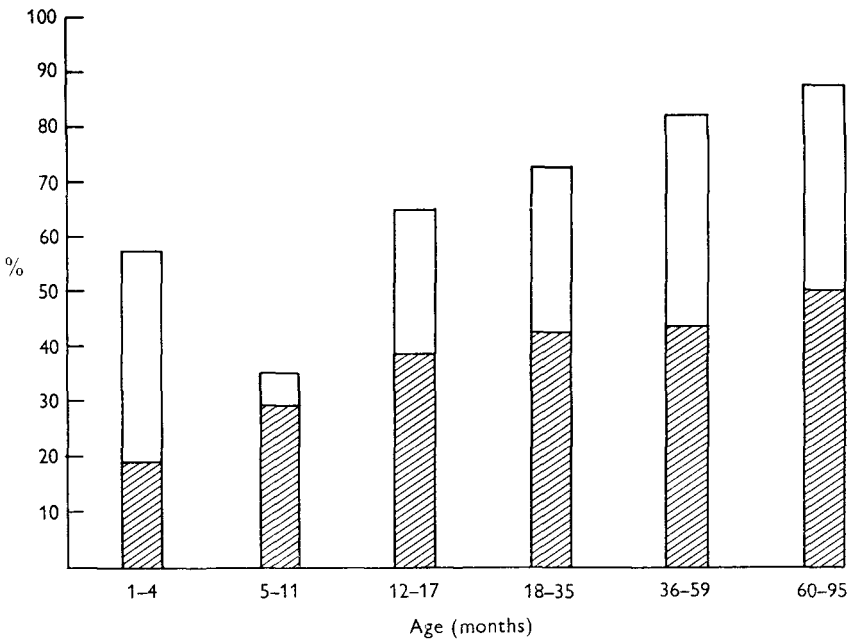

Fig. 2. Correlation of age with the presence of complement-fixing antibody and neutralizing antibody for adenovirus. □, Neutralizing antibody; ▨, complement-fixing antibody.

https://doi.org/10.1017/S0022172400020830 Published online by Cambridge University Press

of any serotype at serum dilutions of 1/5 and 1/80. The neonatal level of passive immunity can be seen to have disappeared after 5 or 6 months of life.

Rate of development of active adenovirus antibodies

Figure 2 shows the percentage of children with demonstrable adenovirus C.F. antibody and neutralizing antibody against any of the adenovirus serotypes 1, 2, 3, 5, 6, or 7. At age 5–11 months, 35·5 % have adenovirus neutralizing antibody for one or more serotypes. The percentage rises with age until by 8 years

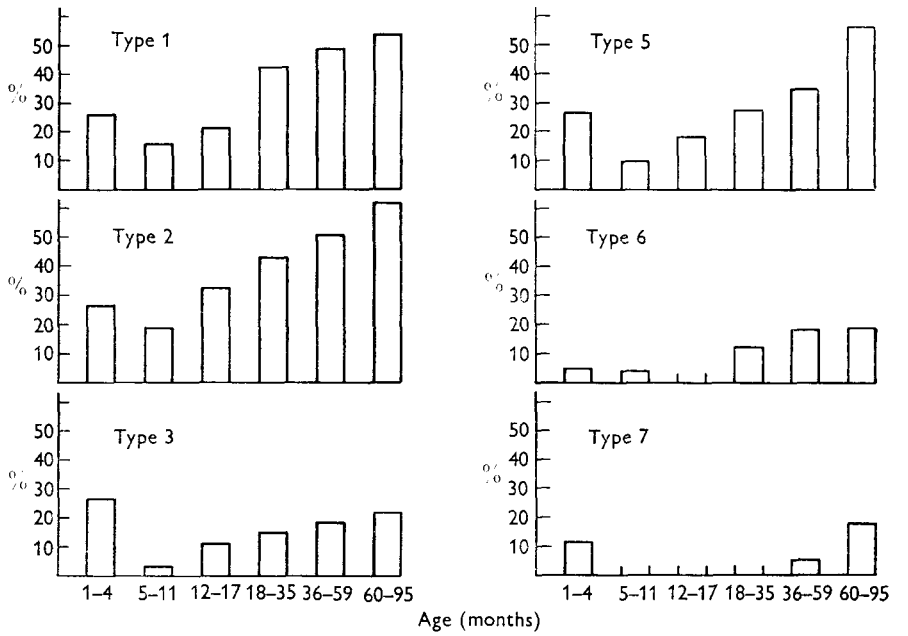

Fig. 3. Correlation of age with the presence of neutralizing antibodies for adenovirus.

Table 1. *Percentages of children with multiple adenovirus neutralizing antibodies*

Total number of adenovirus serotypes neutralized	Age (months)					
	1–4	5–11	12–17	18–35	36–59	60–95
0	42·4	64·5	35·4	27·6	18·2	12·5
1	10·3	19·4	46·4	29·8	27·2	9·4
2	23·1	16·1	17·8	27·6	33·3	43·8
3	11·4	—	—	4·4	6·1	18·7
4	—	—	—	10·6	9·1	6·2
5	—	—	—	—	6·1	9·4
6	3·8	—	—	—	—	—

87·5 % have neutralizing antibody against one or more types. At age 5–11 months, 29·3 % have demonstrable adenovirus C.F. antibody rising to 50 % by 8 years.

Figure 3 shows the percentages of children of various ages with demonstrable neutralizing antibodies against the six adenovirus serotypes. Neutralizing anti-

https://doi.org/10.1017/S0022172400020830 Published online by Cambridge University Press

bodies against serotypes 1, 2 and 5 are much more widely distributed than against types 3, 6 and 7 in the group studied. At age 5–11 months, neutralizing antibody against type 1 is demonstrable in 16·1 % of children, rising to 46·9 % at age 8 years: for type 2 the proportion is 19·4 % at age 5–11 months, rising to 62·5 % at age 8 years. For type 5 the proportion is 9·7 % at age 5–11 months, rising to 56·2 % at age 8 years. Antibody against type 7 was relatively rare. None was detectable in children under 3 years of age.

Table 1 shows the percentage of children at various ages with antibodies against more than one adenovirus serotype.

The number having multiple antibodies is lowest at age 5 months. It increases steadily until, by age 5–8 years, one-third of the children have antibodies against three or more serotypes.

DISCUSSION

The passive acquisition of transplacental adenovirus neutralizing antibody was described by Jordan *et al.* (1958). The data presented in this paper show that the average titre in the new-born child is higher than that in the mother. The ratio of maternal to cord neutralizing antibody levels was approximately the same for adenovirus types 1 and 3.

Active neutralizing antibody for adenovirus serotypes begins to appear in serum specimens early in life and the percentage of children with antibodies against one or more virus types rises quickly with increasing age. Antibodies against adenovirus types 1, 2 and 5 are found more frequently than antibodies against types 3, 6 and 7. As the antibody response in young children is mainly homotypic (Jordan *et al.* 1958), heterotypic response being an antibody recall (Van der Veen & Prins, 1960), it is concluded that infection with adenovirus types 1, 2 and 5 is probably endemic in Sheffield children, just as it is in Cleveland children.

Type 7 antibodies, however, were not encountered in children under 3 years of age. This may be interpreted in two ways. Children over the age of 3 years might be particularly susceptible to infection by this serotype. On the other hand, there may have been no adenovirus type 7 infection in the past 3 years. The latter view is favoured since the presence of antibody in the older children coincided with the presence of this serotype in Sheffield in 1955, described by Tyrrell, Balducci & Zaiman (1956).

From a comparison of this study with two similar studies made in American populations (Huebner *et al.* 1954; Jordan *et al.* 1958) it is evident that adenovirus infection is endemic in all three communities. The Sheffield infection rate is probably higher than that in Cleveland, but lower than that in Washington. The magnitude of endemic infection must be related to the social circumstances of the population studied. Jordan *et al.* (1958) state that the Cleveland study was carried out in a population which 'both at home and school is subjected to the minimal crowding possible in contemporary metropolitan civilization'. This is not true for Sheffield. The social circumstances of the group studied by Huebner *et al.* (1954) are not known.

SUMMARY

The levels of neutralizing antibody against several adenovirus serotypes were determined on 42 paired specimens of maternal and cord serum and on 197 single serum specimens from children up to the age of 8 years.

The antibody distribution indicates that adenovirus infection is endemic in Sheffield just as it has been shown to be endemic in Cleveland and Washington.

We should like to thank Prof. C. P. Beattie for his advice and criticism, Dr C. C. Bowley of the Sheffield Regional Blood Transfusion Centre for paired maternal and cord sera, Dr M. H. Hambling for the provision of adenovirus stock strains and Mr Foster, medical artist, for preparation of the figures.

REFERENCES

Binn, L. N. & Hilleman, M. R. (1960). A guinea pig potency test for adenovirus vaccine. *J. Immunol.* **84**, 20.

Dulbecco, R. & Vogt, Marguerite (1954). Plaque formation and isolation of live lines with poliomyelitis viruses. *J. exp. Med.* **99**, 167.

Huebner, R. J., Rowe, W. P., Ward, T. G., Parrott, R. H. & Betts, J. A. (1954). Adenoidal-pharyngeal-conjunctival agents. A newly recognized group of common viruses of the respiratory system. *New Engl. J. Med.* **251**, 1077.

Jordan, W. S., Badger, G. E. & Dingle, J. H. (1958). A study of illness in a group of Cleveland families. XV. Acquisition of type-specific adenovirus antibodies in the first five years of life—Implications for the use of adenovirus vaccine. *New Engl. J. Med.* **258**, 1041.

Pereira, H. G. (1956). Typing of adenoidal-pharyngeal-conjunctival (A.P.C.) viruses by complement-fixation. *J. Path. Bact.* **72**, 105.

Reed, L. J. & Muench, H. (1938). A simple method for estimating 50 per cent end-points. *Amer. J. Hyg.* **27**, 493.

Tyrrell, D. A. J., Balducci, D. & Zaiman, T. E. (1956). Acute infections of the respiratory tract and the adenoviruses. *Lancet*, ii, 1326.

Van der Veen, J. & Prins, A. (1960). Studies of the significance of the recall phenomenon in the antibody response to adenovirus vaccine and infection. *J. Immunol.* **84**, 562.