stimulation stops. It can be arrested by removing acetylcholine from the bath or by adding atropine or quinidine.

REFERENCES

- Burn, J. H., Gunning, A. J., and Walker, J. M. (1956). *Circulat. Res.*, 4, 288.
 — Vaughan Williams, E. M., and Walker, J. M. (1955). *J. Physiol. (Lond.)*, 128, 277.
 Dawes, G. S., and Vane, J. R. (1951). *Ibid.*, 112, 28P.
 DiPalma, J. R., and Mascarello, A. V. (1951). *Amer. J. Physiol.*, 164, 589.
 Harris, E. J., and Hutter, O. F. (1956). *J. Physiol. (Lond.)*, 133, 58P.
 Hoffman, B. F., and Suckling, E. E. (1953). *Amer. J. Physiol.*, 173, 312.
 West, T. C., Turner, L. D., and Loomis, T. A. (1954). *J. Pharmacol.*, 111, 475.

POLIOMYELITIS ANTIBODY STUDIES IN A GROUP OF LONDON CHILDREN

BY

ANN M. PEACH, M.B., D.C.H.

Research Fellow in Paediatrics, St. George's Hospital,
London

AND

J. A. DUDGEON, M.C., M.D.

Department of Bacteriology, Medical School,
St. George's Hospital, London; now at the Virus Research
Unit, Glaxo Laboratories, Ltd., Greenford

In any virus disease in which subclinical infection takes place the distinction between those who have acquired natural immunity, and therefore have less pressing need for immunization, and those who are still susceptible is difficult. In this respect poliomyelitis stands in marked contrast to measles. It is known that recovery from paralytic poliomyelitis is followed by a prolonged state of immunity in that second attacks of paralysis are rarely encountered, but immunity also develops after non-paralytic and subclinical infection. It has now been clearly established that active immunity in all forms of poliomyelitis infection is closely associated with the presence of circulating antibody; moreover, the incidence of antibody in relation to different age groups has proved to be a reliable guide to the overall immune status of a population.

In the past, serological tests to determine this information were based almost exclusively on the incidence of type 2 antibody, as only one strain (Lansing, type 2 strain) could be handled with any convenience in the laboratory. Since the introduction of tissue-culture methods it is possible to examine sera for antibodies to all three types of poliomyelitis virus and thus gain an overall picture of the pattern of immunity, not only throughout the world but in different sections of a population. From the evidence so accumulated it is now apparent that the incidence of infection with each of the three types is much higher than would be assumed from the incidence of clinical disease, and varies greatly from one part of the world to another (Gear, 1955). For instance, 80% of children in Cairo have by the age of 3 years acquired antibodies to all three virus types. At the same time the evidence of clinical disease is low. In contrast, in an urban population in the United States the same figure of 80% is not reached until the 30–40 age group (Paul, 1955).

The present investigation was undertaken as a preliminary to the introduction of immunization with

poliomyelitis vaccine in the United Kingdom. The purpose of the study was first to determine the incidence of poliomyelitis antibodies (and therefore the inapparent infection rate amongst healthy children); and, secondly, to form some idea of the age at which natural infection begins to take place in such a group. A further purpose was to determine whether the complement-fixation test could be used as a screening test for evidence of past infection.

Materials and Methods

The Study Group.—Ninety children were included in the study during the period March–October, 1955. They were children who had been admitted to the Victoria Hospital for Children with minor medical or surgical complaints. There were approximately equal numbers in each yearly age group from 12 months to 10 years. In no case was there a history of contact with a known clinical case of poliomyelitis, nor of a febrile illness with signs of meningeal involvement. The majority of the children came from the Boroughs of Battersea, Chelsea, and Fulham and, except for three who had been abroad for a short time, had lived nowhere else. The socio-economic background of these children was assessed from the occupation of the father, the intelligence of the parents, and the care given to the child before admission, and was graded as poor, average, or good. The distribution of children from poor, average, and good homes was uniform throughout the age groups.

Sera.—A single specimen of blood was collected from each child. After separation from the clot, sera were divided into two parts; one part for neutralization tests was stored at -65°C ., the other for complement-fixation tests at 4°C .

Virus Strains.—The following strains were used both for complement-fixation and for neutralization tests: Mahoney (type 1), MEF-1 (type 2), Saukett (type 3).

Complement-fixation Test.—In order to economize in material the "drop method" on "perspex" sheets was employed as described for use with poliomyelitis viruses by Svedmyr, Enders, and Holloway (1953) and by Le Bouvier (1953).

Antigens.—These were prepared from monkey-kidney tissue culture and HeLa-cell cultures inoculated with each virus type. The fluids were harvested when cellular degeneration was complete. In the early stages of this investigation complement-fixing antigens were heated to destroy infectivity, but when it became clear that heating resulted in a loss of specificity, subsequent tests were carried out with untreated antigens, special precautions being taken to avoid risk of infection to those carrying out the tests. After inactivation serial twofold dilutions of serum were made in Ca-Mg saline; 4 units of antigen and 2, 2½, and 3 units of complement were used in each test. Fixation was allowed to proceed overnight in the refrigerator. The end-point was taken as the highest dilution of serum producing 50% fixation of antigen with 2½ units of complement.

Neutralization Test.—Preliminary tests were made with the HeLa-cell tissue culture method described by Syverton, Scherer, and Elwood (1954), but, as the results in our hands were at times variable, it was decided to carry out all subsequent tests in monolayer cultures of monkey-kidney epithelium grown in synthetic medium 199 containing 2% horse serum (Youngner, 1954). Monkey-kidney culture tubes prepared seven to eight days previously were examined before use to ensure that sheets of monolayer cells were present. The growth medium was replaced by fresh synthetic medium 199 without horse serum.

Virus.—Types 1, 2, and 3 poliomyelitis viruses from monkey-kidney tissue cultures were diluted so as to contain 100 tissue-culture infective doses previously determined by titration.

Serum Dilutions.—After inactivation serial dilutions of serum were made in medium 199. Screening tests were carried out at 1/4, 1/64, and 1/256; and final tests, if

positive, were made with serial twofold dilution from 1/2 to 1/512 against each virus type.

Neutralization.—Equal quantities of diluted virus fluid of each type were added to each serum dilution and to the necessary control tubes. Serum-virus mixtures were incubated for one hour at 37° C. and then placed in the refrigerator until used for inoculation. Each of two culture tubes was inoculated with 0.5 ml. of the serum-virus mixture. In each test the necessary controls of standard serum and virus were included. After incubation the tests were read on the third and finally on the sixth or seventh day. The end-point was taken as the highest dilution of serum required to neutralize virus in 50% of tubes as determined by the inhibition of the cytopathogenic effect of poliomyelitis virus. The serum dilution was expressed as the final dilution of serum in the serum-virus mixture.

Results

The correlation between complement-fixing and neutralizing antibodies is shown in Table I. The results are based on 82 out of 90 sera, as a number of the earlier specimens had been used up in pilot tests and tests with heated com-

TABLE I.—Correlation of Complement-fixing and Neutralizing Antibodies in 82 Sera

	C.F.T. Positive	C.F.T. Negative
Neutralization test:		
Positive	21	31
Negative	0	30

plement-fixing antigens, subsequently found to be lacking in specificity. It seems clear that so far as these tests go, there is good correlation between the presence of complement-fixing and neutralizing antibody in the same specimen. On the other hand, in 31 cases where the complement-fixation test was negative, the neutralization test was positive. In no instance was a serum with complement-fixing antibody found to be without neutralizing antibody.

The total number of children in each age group who had antibody to any virus type, irrespective of whether that antibody was to one or more virus types, is shown in Table II. It is apparent that the incidence of antibody increases with age, and a definite rise in the numbers occurs about the age of 5 years. The majority (90%) of the children studied over the age of 9 years had antibody to at least one virus type.

The distribution of antibodies in the various age groups is shown in Table III. Those under 5 years had either no antibody or with few exceptions antibody to only one virus

TABLE II.—Incidence of Antibody to One or More Virus Types in Each Age Group

Age:	1	2	3	4	5	6	7	8	9+
No. of children	7	12	11	8	9	11	11	11	10
No. with antibody	2	4	4	4	6	8	9	9	9
Percentage	28	33	36	50	64	72	81	81	90

TABLE III.—Distribution of Poliomyelitis Antibodies in the Various Age Groups

Categories	Age Range			Total
	0-4*	5-8	9-14†	
No. of children examined	38	42	10	90
" " " with no anti-	24	10	1	35
No. of children with antibody to one virus type	11	20	3	34
No. of children with antibody to two virus types	3	8	3	14
No. of children with antibody to three virus types	—	4	3	7

* In 0-4 group there were no children under 12 months.

† In 9-14 group there were no children aged 11 years or more.

type, whereas of those aged 5 and over 44% had antibody to one type, 21% had antibody to two types, and 7 out of 52 (13%) had antibody to all three types.

The incidence of antibody to each individual virus type and combinations of types is shown in Table IV. The numbers are small, but there appears to be a fairly even distribution of antibody to each virus type.

TABLE IV.—Distribution of Poliomyelitis Antibodies to Each Virus Type

No. of Sera with Antibodies	No. of Sera with Antibodies to Single Virus Types or Combinations of Types as Shown						
	1	2	3	1 and 2	1 and 3	2 and 3	1, 2, and 3
55	12	13	9	5	4	5	7
Total	34			14			7

The relationship between the presence of antibodies and socio-economic conditions is shown in Table V. Of the children from poor homes, irrespective of age, 80% had antibodies, while only 50% of those from good homes had antibody.

TABLE V.—Distribution of Antibody in the Three Socio-economic Groups—All Ages

Socio-economic Background	Total	No Antibody	Antibodies Present
Poor	15	3 (20%)	12 (80%)
Average	45	17 (38%)	28 (62%)
Good	30	15 (50%)	15 (50%)

Conclusions

At the start of this investigation laboratory procedures for poliomyelitis were undergoing rapid development, particularly in the use of tissue culture in serological methods. Although the neutralization test had become much easier to perform, technical difficulties still restricted its wider use. On the other hand, the complement-fixation test seemed to offer a number of advantages. High-titre antigens could readily be produced and the test was generally easier and more quickly carried out. However, complement-fixing antibody does not persist for as long as the neutralizing antibody after infection, but it seemed that, as the sera being studied were from children, the complement-fixation test might be useful as a screening test. The first point which emerged was that the use of treated antigens resulted in a loss of specificity in the reaction (Le Bouvier, 1955a, 1955b). Other workers have since found the same (Schmidt and Lennette, 1956). With untreated antigens more clear-cut results were obtained; nevertheless, it is clear that as a screening test for antibody the complement-fixation test is not as reliable as the neutralization test, even when infection can have occurred only a few years previously. The presence of neutralizing antibodies to each of the three types of poliomyelitis virus was therefore taken as evidence of past infection, although, as Salk (1955, 1956) has shown, some individuals without demonstrable antibody have clearly been exposed and infected, as they produce a booster type of response to vaccination.

From the practical point of view, in carrying out neutralization tests, the period for incubating the serum-virus mixtures could probably with advantage be increased to at least three hours at 37° C. Other workers have found this to be more satisfactory.

Although the total numbers in this study group were small the children were a fairly representative cross-section of the child population in the urban areas from which they were drawn. Some idea of the infection rate amongst them can be deduced from the antibody findings. It appears that infection begins to take place at the fifth year of life in the majority, with a shift to a younger age group among poor

children, and to an older age group among children from homes where there is better hygiene and less crowding. The incidence of past infection with each of the three virus types is remarkably uniform, type 3 being a little less common than the others, though not quite so infrequent as Fallon (1956) found in North Wales and in Liverpool.

It is interesting to compare the results of this serological survey carried out in London with two others carried out at the same time, one in the United Kingdom and the other in Morocco. Horstmann (1955) found that amongst Moroccan children in Casablanca the incidence of antibody to all three virus types had reached 80% by the age of 5 years. The rarity of clinical disease, except in those under 2 years of age, was an indication of the widespread dissemination of virus under these conditions. Fallon (1956) carried out an investigation in the rural district of Bettws-y-Coed in North Wales with a population of 780 inhabitants in which no cases of paralysis had been reported since 1912. He found a surprisingly high incidence of antibody to all three virus types in various combinations in children over the age of 4 years. In fact, the incidence of type 1 antibody in Bettws-y-Coed was slightly higher than in Liverpool or in Cairo.

Although the poliomyelitis immune state of any one individual can be learned only from serological tests, the pattern of poliomyelitis infection rates within a community can be drawn from cross-section studies such as the present one. It would suggest that amongst the children from the areas studied it is those under 5 years who would benefit most at the present time from immunization against poliomyelitis, although these findings do not necessarily reflect the immune status throughout the country.

Summary

Sera from 90 London children aged 12 months to 10 years were examined for the presence of antibodies to the three types of poliomyelitis virus.

Tests were carried out for both complement-fixing and neutralizing antibodies, and both methods are described.

As a screening test for antibody, the neutralization method in tissue culture was found to be the more reliable guide to past infection.

In the small group of children studied, subclinical infection began to take place at about the fifth year of life.

The incidence of past infection with each of the three virus types was fairly uniform, type 3 being slightly less common amongst the younger children.

We thank the consultant physicians and surgeons of the Victoria Hospital for Children for permission to investigate these patients under their care, and the parents of the children for their valuable co-operation. We are grateful to Dr. W. Wood, of Glaxo Laboratories Ltd., for kindly supplying virus strains, and virus fluids for antigen, and for a great deal of help. We also thank Miss E. M. Howard and Mr. G. T. Hawkins for their expert technical assistance. One of us (A. M. P.) is in receipt of a research grant from the Endowment Fund of St. George's Hospital. One of us (J. A. D.) was in receipt of a grant from the Medical Research Council and from the National Fund for Poliomyelitis Research.

REFERENCES

- Fallon, R. J. (1956). *Lancet*, **1**, 65.
 Gear, J. H. S. (1955). *Wld Hlth Org. Monogr. Ser.*, No. 26, p. 31. Geneva.
 Horstmann, D. M. (1955). *Ann. N.Y. Acad. Sci.*, **61**, 956.
 Le Bouvier, G. L. (1953). *Brit. J. exp. Path.*, **34**, 300.
 — (1955a). Personal communication.
 — (1955b). *Lancet*, **2**, 1013.
 Paul, J. R. (1955). *Wld Hlth Org. Monogr. Ser.*, No. 26, p. 9. Geneva.
 Salk, J. E. (1955). *Ann. N.Y. Acad. Sci.*, **61**, 1023.
 — (1956). *Amer. J. publ. Hlth*, **46**, 1.
 Schmidt, N. J., and Lennette, E. H. (1956). *J. exp. Med.*, **104**, 99.
 Svedmyr, A., Enders, J. F., and Holloway, A. (1953). *Amer. J. Hyg.*, **57**, 60.
 Syverton, J. T., Scherer, W. F., and Elwood, P. M. (1954). *J. Lab. clin. Med.*, **43**, 286.
 Youngner, J. S. (1954). *Proc. Soc. Exp. Biol. (N.Y.)*, **85**, 202.

DENTAL EXTRACTION IN HAEMOPHILIA AND CHRISTMAS DISEASE

BY

JOHN A. ORR, L.D.S., H.D.D.

Oral Surgery Department, Glasgow University Dental
School, Dental Hospital, Glasgow

AND

A. S. DOUGLAS, M.B., B.Sc., M.R.C.P.
M.R.C.P.Ed., F.R.F.P.S.

University Department of Medicine, Royal Infirmary,
Glasgow

The management of the haemophiliac presents many difficulties, and not least among these is the problem of dental extraction. Haemophilia is a rare disease, the incidence in the population in the West of Scotland being 1 per 30,000–35,000. In contrast to this low incidence is the frequency with which these sufferers require hospital management; amongst the commonest causes of admission is the necessity for tooth extraction. Most haemophiliacs show great reluctance in seeking dental attention. The fear of dental extraction is of course well justified; unfortunately this dread of the dentist prevents the proper care of the teeth by conservative methods, and as a consequence their teeth are commonly neglected. On several occasions we have seen haemophiliacs of about 20 years of age requiring complete clearance of teeth. Many of the teeth are represented merely by shells submerged in the gum margin, and consequently periodontal sepsis is frequent.

It is the object of this communication to report our experiences in the management of dental extractions in patients with haemophilia or the closely allied condition Christmas disease.

Methods

Thirty-two dental operations, involving the removal of 57 teeth, were carried out on 10 patients; nine of these were haemophiliacs, lacking antihæmophilic globulin, and the tenth was a patient with Christmas disease, lacking the Christmas factor (Biggs *et al.*, 1952). The number of dental operations performed on each patient varied from one to seven, depending on how many teeth required extraction. The important features of these dental operations are summarized in Table I. The patients were all admitted to hospital; they were warned that the measures to be adopted were unlikely to prevent hæmorrhage completely, but that any consequent bleeding would not be serious in its extent.

Number of Extractions at Each Operation.—The number of teeth extracted at each operation varied from one to four, and may be summarized as follows: at 14 operations, one tooth; at 12 operations, two teeth; at 5 operations three teeth; and at one operation, four teeth extracted. As a consequence of experience it was decided that, in general, only one or two teeth should be extracted at any one operation. When adjacent teeth were carious it was considered justifiable to extract two at one sitting. The extraction of more than two teeth resulted in much troublesome hæmorrhage. These observations confirmed the opinion of White and Mallett (1949). The hæmorrhage which follows the extraction of large numbers of teeth carries risk to life and is alarming to the patient. It is not practicable to provide maintained hæmostatic levels of antihæmophilic globulin over long periods with available sources of supply. The practice of extracting large numbers of teeth after alleged correction of the hæmostatic defect by administration of plasma, plasma products, or whole blood should be abandoned. The teeth extracted were all