Immunity of children to diphtheria, tetanus, and poliomyelitis

D BAINTON, MARIE FREEMAN, D I MAGRATH, F SHEFFIELD, J W G SMITH

British Medical Journal, 1979, 1, 854-857

Summary and conclusions
A survey of titres of diphtheria and tetanus antitoxins and of antibodies to polioviruses in the sera of 281 schoolchildren aged 15, 11, and 7 years showed that high immunisation rates can evoke protective concentrations of tetanus antitoxin in 98% of children and protective levels of the antibodies to diphtheria and all three types of poliomyelitis in 85% of children. Reinfocing immunisation at school entry appeared to be necessary to maintain adequate titres of diphtheria antitoxin in children up to 15 years of age, not essential to maintain adequate titres of tetanus antitoxin, and to have little effect on the titres of antibodies to poliomyelitis.

Introduction
In Britain and many other countries preventive medicine has virtually eradicated diphtheria and poliomyelitis and greatly reduced the incidence of tetanus. These successes of immunophylaxis are continuously and precisely recorded epidemiologically but, except in the short term of a vaccine trial, are only rarely related to the serological phenomena on which they depend. Serological surveys, particularly on young people, are notoriously difficult to organise owing to problems associated with selecting population samples truly representative of the cohorts of interest, collecting blood samples, and limited facilities for titrating antibodies. Nevertheless, as immunisation programmes approach their objectives and natural infections become rare serological surveys become ever more necessary, since they are the only indicators of the continuing effectiveness of an immunisation schedule and the need to modify immunisation schedules or vaccine potency.

Recently in the county of Avon special conditions provided valid reasons for collecting blood from three precisely defined population samples and estimating the concentrations of all the serum antibodies, other than those to Bordetella pertussis, that may be expected consequent on primary immunisation in infancy and reinforcing immunisation at school entry. The samples consisted of children aged 15, 11, and 7 years and the results obtained thus indicated the immune states of the cohorts from which the samples were drawn some 10, six, and two years after the scheduled time for reinforcing immunisation.

Patients and methods
Children—Three samples, each comprising 120 children or about 1% of the available population, were selected at random, one sample being taken from each cohort born in 1961, 1965, and 1969 in the area of the Avon Area Health Authority. The study was publicised by local newspapers, radio, and television, and the parents of each selected child were approached by post of their child’s inclusion in a sample and asked to co-operate. During November 1976 medical staff of the school health service visited the home of each selected child, provided the parents with any further information that they requested, and obtained from the child, by venepuncture, a 10 ml blood sample. Each blood sample was matched with the record card of that child and sent to the laboratory for separation of serum. The immunisation history of each child was obtained from the school health service records.

Titres of diphtheria antitoxin—Diphtheria antitoxin was measured by neutralisation tests in microtitre plates.1 HeLa cells and a toxin test dose of 2 TCD₅₀ were used in all assays. The British standard for diphtheria antitoxin was used as the reference material. The results were expressed in IU/ml serum.

Screening for tetanus antitoxin—Each serum sample was initially screened for 0.1 IU tetanus antitoxin/ml. Sera containing less than this concentration were screened for 0.01 IU/ml. Screening was done by neutralisation tests in mice according to the method of the European Pharmacopœia except that, to achieve the requisite sensitivity, the tests were conducted at the Lp/400 rather than the Lp/10 level. The British standard for tetanus antitoxin was used as the reference material.

Titres of poliovirus antibodies—Poliovirus antibodies were measured by neutralisation tests in microtitre plates containing cultured HeP₂C cells. Each serum sample was diluted to provide a dilution series in fourfold steps from 1/4 to 1/256, and from this series three subordinate series were drawn. To each dilution of these three series were added between 30 and 200 TCD₂₀ of the three Sabin attenuated polioviruses, one virus type being added to each dilution series. The mixtures of serum and virus were incubated at 35°C for two hours and then added to the wells. The plates were incubated at 35°C for seven days, and at the end of this time the cultures were inspected for cytopathic effect. The appropriate British standard for antipoliovirus serum was used as a reference material. The titres were expressed as the reciprocal of the serum dilution that neutralised the virus in 50% of the wells. Dividing the titres by 35, 21, and 73 in the cases of types 1, 2, and 3 respectively gives a good approximation of the antibody titres in international units.

Replicate samples—Serum samples from a random 10% of the children were split into two aliquots, one being identified with the number and name of the child and the other labelled with a number and a fictitious name. In each case both aliquots were submitted to the laboratory without indication of their common origin and all five antibodies assayed in both. The results included here were those obtained from the truly labelled aliquots, but in every case comparability of the results obtained with each pair was satisfactory.

Results
The three samples of 120 children contained 31 children who were known to have left Avon and a further 18 who could not be traced despite repeated attempts. Of the remaining 311 children available for the study, 19 children or their parents refused to co-operate, leaving 292 participants, an overall response rate of 94%. In only one case was the volume of blood obtained insufficient.

Immunisation records were available and complete for 246 of the 291 children from whom satisfactory blood samples were obtained, available but incomplete for 36 children, and unavailable for nine.

These data summarise the immunisation histories of the sample. If an unqualified record of immunisation is assumed to signify the administration of all the vaccines and doses that were required by the national immunisation schedule, 171 children had received both primary and reinforcing immunisation and 93 had probably received only primary immunisation. Because of a steady increase in the uptake of reinforcing immunisation most of the children who had received
Immunisation histories of 291 children from whom satisfactory blood samples were obtained

<table>
<thead>
<tr>
<th>Year of birth</th>
<th>1961</th>
<th>1965</th>
<th>1969</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary immunisation and reinforcement</td>
<td>35</td>
<td>53</td>
<td>83</td>
<td>171</td>
</tr>
<tr>
<td>Primary immunisation without reinforcement or record of reinforcement</td>
<td>44</td>
<td>35</td>
<td>14</td>
<td>93</td>
</tr>
<tr>
<td>No primary immunisation or record of primary immunisation</td>
<td>17</td>
<td>8</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>96</td>
<td>99</td>
<td>291</td>
</tr>
</tbody>
</table>

only primary immunisation were in the 1961 and 1965 samples and almost all of the children in the 1969 sample were fully immunised. The 27 children who were incompletely immunised, not immunised, or without immunisation records were originally thought likely to provide informal but useful baseline values with which the antibody titres of their immunised peers might be meaningfully compared. Eleven of these children, however, were found to have antibodies to all vaccine antigens and so must almost certainly have been immunised.

Throughout this report the results of the antitoxin and antibody assays are recorded as frequency distribution histograms. Each serum titre indicates the immunisation history of the child from whom the sample was obtained: (1) fully immunised—a child with records of both primary and reinforcing immunisation; (2) primarily immunised only—a child with a record of primary immunisation but lacking a record of reinforcing immunisation; (3) others—a child recorded as not having been immunised or a child without records.

Fig 1 shows the diphtheria antitoxin titres of children in the three samples. There was a preponderance of low titres in the 1961 sample, intermediate titres in the 1965 sample, and high titres in the 1969 sample; this pattern applied not only to each distribution as a whole but also to each distribution in respect of the fully immunised children alone. The low titres in the 1961 distribution were largely contributed by children who had had to receive reinforcing immunisation or whose immunisation records were doubtful, and the high titres in the 1969 sample were almost wholly contributed by children who had received reinforcing immunisation within the two years before blood sampling.

If an antitoxin titre of 0·01 IU/ml—that is, about three times the concentration associated with Schick conversion—is considered to indicate adequate immunisation, the numbers of thoroughly protected children in the 1961, 1965, and 1969 samples were 58 (60%), 70 (73%), and 92 (93%) respectively. The 71 children with antitoxin titres below 0·01 IU/ml, and who may be considered to have been inadequately protected, comprised 17 who had been fully immunised, 38 who had received only primary immunisation, and 16 who were without adequate records.

Fig 2 shows the tetanus antitoxin titres in the three samples. The method used for screening for tetanus antitoxin gave titres within three ranges, but regardless of immunisation history or time since the last immunisation almost all titres were in the highest range—that is, over 0·1 IU/ml. If 0·01 IU tetanus antitoxin/ml is taken to indicate adequate immunisation there were only eight children, none of whom had been fully immunised, who may be thought to have been inadequately protected.

Figs 3, 4, and 5 show the poliovirus antibody titres in the three samples of children. In all three samples the median titres of type 1 antitoxin were 1·0 IU/ml. The titre of 1·0 IU/ml is taken to indicate adequate immunisation there were only eight children, none of whom had been fully immunised, who may be thought to have been inadequately protected.
and type 2 antibodies were higher than the median titres of type 3 antibody, the overall median titres for the three types being 1/48, 1/45, and 1/22 respectively. In the 1961 and 1965 samples there were enough children who had received only primary immunisation to permit a meaningful comparison of the antibody titres of the fully immunised children with those of children who had received only primary immunisation against poliomyelitis. In each distribution the contributions to each titre range made by the fully and partly immunised children were roughly comparable and there was little evidence that reinforcement had induced higher titres of poliomyelitis antibodies. The only exception was the disproportionately larger number of fully immunised children in the 1965 sample with type 2 antibody titres in the highest range.

If in the cases of all three serotypes an antibody titre of 1/8 is taken to indicate adequate immunisation there were, in the 1961, 1965, and 1969 samples respectively, 93 (97%); 89 (92%); and 95 (86%) children adequately immunised against type 1 infection; 93 (97%); 94 (98%); and 99 (100%) children adequately immunised against type 2 infection; and 85 (89%); 81 (84%); and 93 (94%) children adequately immunised against type 3 infection.

Fig 6 shows the overall patterns of antibodies in the sera of children in the three samples. In this figure the abscissae represent the numbers of different antibodies (diphtheria antitoxin t 0·01 IU/ml, tetanus antitoxin t 0·01 IU/ml, and antibodies to polioviruses types 1, 2, and 3) in sera of children born in 1961, 1965, and 1969. Key as in fig 1.

Discussion

An antibody survey such as this cannot provide an exact measure of either the overall immunity of the studied samples or the overall efficacy of an immunisation programme. Under-estimation of immunity almost certainly arose from our need to equate protection with arbitrary antibody titres and our inability to assess the protective role of immunological memory. Con-
readily established in most children by primary immunisation alone, and the prime benefit of reinforcement at school entry may be to prolong immunity through adolescence into adulthood. In the case of poliomyelitis, reinforcing immunisation was largely without effect, probably on account of exclusion by pre-existing antibody. Thus it might be argued that, except in the case of immunity to diphtheria, the benefits of reinforcing immunisation are chiefly the immunisation against tetanus and poliomyelitis of children who have inadvertently missed primary immunisation.

Extrapolation from the samples to the cohorts from which they were drawn provides estimates of the percentages of children who, by the criteria of this study, may be considered to have been adequately protected. Thus in the 1969 cohort, in which the best immunisation coverage was achieved (84% of the children were fully immunised and a further 14%, had received primary immunisation, although not reinforcement), the estimate of those protected from tetanus was 98%, and of those protected from diphtheria and all three types of poliomyelitis 85%. In view of the excellence of the protection from tetanus and the additional protection provided by the herd in the cases of the communicable infections, it seems unreasonable to suppose that more rigorous implementation of an immunisation programme would provide anything other than marginal additional benefit.

We thank the children for the blood samples, and their parents for allowing them to participate; Mr R Youens and Mrs Vivienne Miller and staff of the child health service of the Avon AHA; the doctors of the Avon AHA for performing the venepunctures; Dr Suzanne Clarke, of the Bristol PHLS Laboratory, for separating the sera; and Miss Janet Bootman, Mrs Anne Johns, Miss Moira McVicar, and Miss Johanna Watkins for skilled technical help.

References

(Accepted 8 February 1979)

Campylobacter colitis

M E LAMBERT, PHILIP F SCHOFIELD, A G IRONSIDE, B K MANDAL

Summary and conclusions
Eleven consecutive patients with diarrhoea from whose stools campylobacter were isolated were investigated by sigmoidoscopy and rectal biopsy. Eight had definite proctitis, and in seven biopsy specimens were abnormal with histological changes ranging from non-specific colitis to gross colitis with goblet-cell depletion and crypt-abscess formation. Nine of the patients passed blood in their stools, and in all but one abdominal pain was a feature of the illness.

Severe campylobacter colitis may be clinically, sigmoidoscopically, and histologically difficult to differentiate from ulcerative colitis and is a differential diagnosis in acute colitis.

Introduction
Campylobacter has only recently been recognised as a cause of diarrhoea. Skirrow\(^1\) reported that this organism could be isolated by selective culture of the faeces of over 7% of unselected patients with diarrhoea but not from control samples from subjects without diarrhoea. Other studies\(^2\)–\(^9\) have confirmed these findings. During 1977 1513 reports of the isolation of campylobacter from the faeces of patients were received by the Communicable Disease Surveillance Centre (Public Health Laboratory Service), and out of 1336 cases analysed, 95% of the patients were suffering from diarrhoea.\(^10\)

The clear correlation between symptomatic diarrhoea and the isolation of campylobacter leaves little doubt that it is potentially pathogenic, though “carrier states” probably exist.

The concept of colonic disease resulting from salmonella infection, which was previously regarded as causing “enteritis,” has recently gained wide acceptance.\(^11\) In view of the close similarity between the clinical features of salmonella and campylobacter infections we carried out the present study to determine whether similar colonic changes occurred in patients with diarrhoea due to campylobacter.

Patients and methods
Eleven patients (three female, eight male) presented during a six-month period with a diarrhoeal illness and campylobacter in their faeces. They were aged 14-80 years (mean 46 years), and 10 warranted admission for inpatient management. Sigmoidoscopy was carried out in all these cases and rectal biopsy specimens taken; in eight cases this was done shortly after admission. Three patients who did not undergo sigmoidoscopy on admission were found to have campylobacter in their stools only after the diarrhoea hadsettled; sigmoidoscopy and biopsy were performed as soon as the diagnosis was made, so in these cases the result represents a resolving or resolved condition. A barium enema was thought to be justified in two patients. Filtrates of faecal suspensions were cultured at 43°C on Oxoid BA base No 2 with 7% lysed horse blood plus (final concentrations) vancomycin 10 μg/ml, polymixin B sulphate 2-5 IU/ml, and trimethoprim lactate 5 μg/ml, in an atmosphere of 5% oxygen, 10% carbon dioxide, and 85% hydrogen.

CLINICAL FEATURES
The patients presented with diarrhoea or abdominal pain, or both. In most cases the duration of diarrhoea before presentation was...