J. Hyg., Camb. (1963), **61**, 425
Printed in Great Britain

A trial of reinforcing doses of diphtheria toxoid absorbed through the buccal mucosa

*A Report by the Public Health Laboratory Service and the Manchester Education Committee School Health Service**

(*Received* 21 *May* 1963)

The administration of diphtheria toxoid without injection has been tried by nasal instillation (Jensen, 1937), inhalation (Bousfield & King-Brown, 1938), application to the skin (Fraser, Davey & Halpern, 1940; Bousfield, 1944) and orally in a tablet which was swallowed (Bousfield, 1945; Greenberg & Fleming, 1950). These methods were either ineffective or gave rise to serious side-effects (Lancet, 1941) which precluded their use. Bousfield (1945) and Masucci, Gold & DeFalco (1948) showed that toxoid administered in a lozenge which was allowed to dissolve slowly in the mouth was free from side-effects and produced an antitoxin response in most persons with a history of previous immunization or an attack of diphtheria. Cockburn and his colleagues, in a preliminary study using lozenges containing diphtheria toxoid and hyaluronidase, demonstrated a satisfactory antitoxin response in a small group of adults (Cockburn, Bradstreet, Bailey & Ungar, 1961).

The purpose of this trial was to find out whether these lozenges were a satisfactory means of reinforcing immunity in children aged 10–12 years who had been previously immunized by parenteral injection, and to compare the antitoxin response with that following the injection of formol toxoid.

MATERIALS AND METHODS

The children

The trial took place in three schools in Manchester. The children were between 10 and 12 years old and had records of primary immunization with at least two injections of diphtheria prophylactic with or without subsequent reinforcing doses, the last dose having been given more than 12 months before the commencement of the trial.

One-hundred and eighty children took part in the trial and were divided into three groups of sixty each by means of random sampling numbers. One group received formol toxoid by injection; another group two lozenges at an interval of

* The trial was designed and the results analysed by Dr W. C. Cockburn (present address: Division of Communicable Diseases, World Health Organisation, Geneva) and Dr N. S. Galbraith, Central Public Health Laboratory, Colindale, London, N.W. 9. Dr C. Metcalfe Brown, Dr E. M. Jenkins, Dr M. C. Davitt, Dr S. J. Jaron and Dr J. M. McCarthy, Manchester, carried out the field investigations. The antitoxin titrations were performed by Dr C. M. P. Bradstreet and Miss E. M. Bailey, Colindale. The diphtheria toxoid lozenges were prepared by Dr J. Ungar, Glaxo Research Laboratories.

1 week; and the third group three lozenges, the first two at an interval of 1 week and the third 1 month later.

Blood specimens were collected from the children immediately before the administration of the toxoid and again between 2 and 3 weeks after the injection or the last lozenge. One year later a third blood specimen was obtained from as many children as possible whose initial antitoxin titres were equal to or less than 0·1 unit per ml. and also from a sample of children in the lozenge groups whose titres were between 0·1 and 1·0 unit per ml.

The toxoid

Each lozenge contained 1500 Lf. of diphtheria toxoid and 1500 units of hyaluronidase (Cockburn *et al.* 1961). The lozenges were given to the children at school under supervision and the children were instructed to allow the lozenges to dissolve slowly in their mouths.

The formol toxoid, in a dose of 25 Lf., was given by intramuscular injection.

The antitoxin titrations

The blood samples were centrifuged within 24 hr. after collection and the sera were stored at $-20°$ C. until required. The titrations were done on the paired samples when all the second sera had been collected. The titrations of the second sera were repeated with the corresponding third sera in the children who were bled again 1 year after immunization. The antitoxin was titrated by the method previously described (Cockburn *et al.* 1961).

Table 1. *Number of children immunized in the trial and number of sera examined*

Immunization group	Number of children: Immunized	First and second sera examined	Selected for bleeding 1 year after immunization	Third sera examined
Injection	60	46	20	12
Two-lozenge	60	52	68	44
Three-lozenge	60	36		
Total	180	134	88	56

RESULTS

One hundred and eighty children were included in the trial, and paired blood samples were examined from 134 (Table 1). In forty children paired sera were not obtained; in two the serum containers broke in transit; in one the serum control gave a positive result; in one the guinea-pig died and there was insufficient serum to repeat the test; and in two children with the same name and initials the specimens could not be distinguished.

Of the 134 remaining children, 67 were boys and 67 girls; 46 children received formol toxoid by injection, 52 received two lozenges and 36 received three lozenges.

https://doi.org/10.1017/S0022172400021045 Published online by Cambridge University Press

Eighty-eight of the children were selected for bleeding 1 year after immunization, and sera were obtained from 56 of them (Table 1). There were no reports of any reactions after the administration of the lozenges and the children did not find them unpleasant to take.

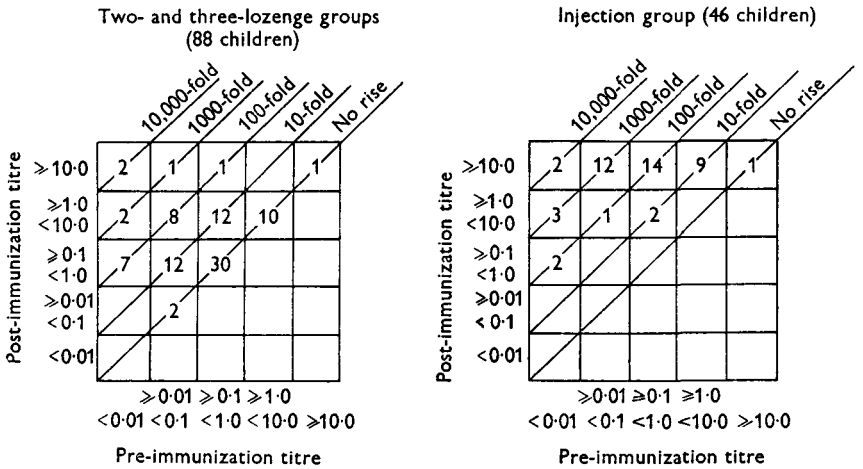

Fig. 1. Antitoxin titres before and 2–3 weeks after immunization.

Table 2. *Antitoxin titres in fifty-six children from whom serum samples were obtained 1 year after immunization*

Immunization group and number immunized	Serum sample	Antitoxin titre (units per ml.) < 0·01	⩾ 0·01 < 0·1	⩾ 0·1 < 1·0	⩾ 1·0 < 10·0	⩾10·0
		Number of children				
Injection (12 children)	Pre-immunization	4	7	1	0	0
	2–3 weeks after immunization	0	0	1	3	8
	1 year after immunization	0	1	4	6	1
Two-lozenge and three-lozenge (44 children)	Pre-immunization	7	15	22	0	0
	2–3 weeks after immunization	0	1	25	15	3
	1 year after immunization	0	7	30	5	2

Vertical line indicates antitoxin titre below which immunization is thought to be necessary (Barr & Cunliffe, 1954).

Fig. 1 shows the antitoxin titres before and 2–3 weeks after immunization in the three groups of children. As the antitoxin response in the two-lozenge and three-lozenge groups was similar they have been combined in the figure. Of eleven children with initial titres of less than 0·01 unit per ml., all had titres of 0·1 unit or more after immunization. Only two of thirty-four children with initial titres less than 0·1 unit failed to respond to immunization by lozenge.

https://doi.org/10.1017/S0022172400021045 Published online by Cambridge University Press

The antitoxin response in the injection group was greater than in the lozenge groups. There was no difference in the antitoxin response between boys and girls or between children from different schools. The antitoxin response was greater in children with low initial titres than in those with high titres. This difference was more noticeable in the lozenge groups than in the injection group.

The antitoxin titres in fifty-six children from whom a blood sample was obtained 1 year after immunization are shown in Table 2. In the injection and the lozenge groups the titres had fallen after 1 year, but in all the children the titres were equal to or greater than 0·01 unit per ml. Seventeen of the children tested in the lozenge groups with initial titres greater than 0·01 unit per ml. had no rise in titre after immunization; none of them showed any rise a year later, but in none did the titre fall below 0·01 unit.

DISCUSSION

The Schick test, which is often used to determine immunity or susceptibility to diphtheria, does not correspond to a precise antitoxin titre, but is usually negative when the titre is greater than 0·004 unit per ml. and positive below this (Greenberg & Roblin, 1949; Barr, Stamm & Stevens, 1957). Most of the children in the trial would probably have been Schick negative before immunization; after immunization they would all have been Schick negative, as none of them had post-immunization titres of less than 0·01 unit per ml.

Barr & Cunliffe (1954) and Barr *et al.* (1957) considered that the Schick test titre of antitoxin was not satisfactory as a measure of immunity in an artificially immunized community, and suggested that a titre of less than 0·01 unit per ml. indicated that further immunization was necessary.

Eleven children in the lozenge groups had initial titres of less than 0·01 unit and all had a 100-fold or greater rise in titre after immunization (Fig. 1). Thirty-two (94 %) of the thirty-four children in lozenge groups with initial titres below 0·1 unit had an antitoxin response. It appears, therefore, that the lozenges were a satisfactory means of reinforcing immunity in this group of 10–12 year old children.

There was little difference in the response of the two-lozenge and three-lozenge groups. The response in both was less than in the injection group, but the lozenges produced a substantial rise in antitoxin titre in children with low initial titres.

The oral route has considerable administrative advantages over injection, and children would probably accept oral immunization more readily. The results of this trial suggest that, in children who have been previously immunized by injection, the reinforcing injection usually given between 10 and 12 years of age might be replaced by a course of two lozenges at an interval of 1 week. The lozenges might also be a valuable means of reinforcing the immunity of a large number of children quickly during an epidemic of diphtheria.

The lozenges must be administered under supervision to ensure that the children allow them to dissolve slowly in their mouths. If they are swallowed they are likely to be ineffective.

SUMMARY

Diphtheria toxoid lozenges were given to eighty-eight children aged 10–12 years who had previously been immunized by parenteral injection, and the antitoxin titres before and after administration were compared with those in a similar group of forty-six children who received formol toxoid by injection.

The results showed that a course of two lozenges at an interval of 1 week was a satisfactory means of reinforcing immunity in the children. The antitoxin response was less than that after injection, but a substantial response occurred in the children with low initial titres.

It is suggested that the lozenges might replace an injection as a means of reinforcing immunity in children aged 10–12 years and that they might be a valuable means of rapidly reinforcing the immunity of a large number of children in an epidemic.

The lozenges must be given under supervision to ensure that the children allow them to dissolve slowly in their mouths.

We should like to thank Dr J. D. Abbott of the Public Health Laboratory, Manchester, for separating the sera.

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