DIPHTHERIA IN THE IMMUNIZED WITH OBSERVATIONS ON A DIPHTHERIA-LIKE DISEASE ASSOCIATED WITH NON-TOXIGENIC STRAINS OF *CORYNEBACTERIUM DIPHTHERIAE*

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The initiation in this country of a national campaign to reduce the incidence of diphtheria by mass immunization focused attention on attacks of diphtheria occurring in inoculated persons. In 1939 the Preventive Medicine Committee of the Medical Research Council began an investigation of this problem. Detailed observations were made in Newcastle, Gateshead and Dundee, where outbreaks of a severe form of diphtheria were occurring, caused by the *gravis* type of *Corynebacterium diphtheriae* and affecting a high proportion of inoculated persons (Hartley, Tulloch, Anderson, Davidson, Grant, Jamieson, Neubauer, Norton & Robertson, 1950). A number of cases of diphtheria in the inoculated were also reported from Cardiff; in this city and neighbourhood the *intermedius* type was responsible for the majority of infections. An investigation in Cardiff of diphtheria in the inoculated, for comparison with the observations made on Tyneside and at Dundee, was therefore thought likely to be of interest. It was carried out in co-operation with Sir Percival Hartley, who kept us informed of his findings and of those of his colleagues elsewhere. Although the investigation was completed in 1944 this report has been delayed to allow the prior publication of the more comprehensive observations on Tyneside and at Dundee upon which it was based. There may be some inadequacies, due to the delay, and it is particularly regretted that the non-toxigenic strains of *C. diphtheriae* to be described are no longer available for further study.

PROCEDURE AND METHODS

The patients investigated included all those admitted with diphtheria to Cardiff City Isolation Hospital between March 1943 and October 1944 in whom there was a history of previous immunization, *C. diphtheriae* being found in the nose and throat swabs taken on admission. As a control, observations were also made on a small number of persons suffering from diphtheria who had not been immunized previously. These cases were chosen at irregular intervals during the investigation and may represent an undue proportion of mild cases. Later, observations were extended to include all patients admitted with diphtheria in whom there was a history of a previous attack.

Nose and throat swabs were taken from each patient on admission, and from

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them cultures were made on Loeffler slopes, on Hoyle's medium and on blood-agar plates. Next day the slopes and the Hoyle's plates were examined for the presence of *C. diphtheriae*, the plates being examined again after 48 hr. incubation. The presence on the blood-agar plates of haemolytic streptococci or any other organism of pathogenic significance was noted. Films were made directly from the throat swabs and examined for Vincent's organisms. All strains of *C. diphtheriae*, obtained in subculture by picking from single colonies, were typed by noting the appearance of the colonies on Hoyle's medium, the type of growth in broth, and by testing for the fermentation of glucose, saccharose and starch. A virulence test was carried out by inoculating a guinea-pig subcutaneously with a suspension of organisms, obtained from an overnight culture on a Loeffler slope by washing off the whole of the growth with broth. Animals were kept under observation for at least 2 weeks before being discarded as negative. The serological grouping of each strain of *C. diphtheriae* was also very kindly undertaken for us by Prof. D. T. Robinson.

On admission, and before any antitoxin was administered, a sample of blood was withdrawn from a vein. These samples were collected using sterile all-glass syringes and screw-capped bottles, which were supplied by the laboratory and had been carefully washed and sterilized in the hot-air oven at 160° C. for 1 hr. In order to avoid the possibility that the samples might be contaminated with the highly concentrated antitoxin used therapeutically, strict instructions were given that blood should only be taken with this specially prepared apparatus, which should never be used for the administration of antitoxin. The importance of such precautions was emphasized by Hartley *et al.* (1950). The serum was separated from each sample of blood, and the amount of diphtheria antitoxin present estimated by the intracutaneous injection of guinea-pigs, as described in detail by Hartley and his colleagues. In this paper the results of the estimations are minimal values; the actual amounts of antitoxin were greater, but less in each case than the next higher value tested, which was usually twice the first. All these estimations were very kindly carried out for us by Sir Percival Hartley.

A second sample of blood was withdrawn during convalescence, the same precautions being observed, and the antitoxin in the serum estimated. These later samples were taken between the 5th and 19th weeks, usually about the 6th to 8th week. It was considered that in these estimations the antitoxin measured was mostly that formed by the patient, because Hartley *et al.* showed that by the end of 4 weeks the antitoxin administered therapeutically had fallen to a low level in the serum.

Information about each patient, collected on a special form by the Medical Superintendent of the Isolation Hospital, included details of the patient, with the dates of onset of the disease and admission to hospital, the site and degree of membrane formation, a clinical estimate of the severity of the disease, the dose of antitoxin administered and the occurrence of complications. The public health department supplied details regarding previous attack of the disease, immunization history, the dates and dosage of prophylactic, what prophylactic was used and its maker, and the result of any earlier or later Schick tests.

RESULTS

A small number of cases on which observations were made, finally diagnosed on clinical grounds as 'carriers', were excluded from the final analysis. There remained a series of eighty-one cases investigated. The routine testing for virulence of every strain of *C. diphtheriae* isolated on admission to hospital revealed the surprising fact that in nineteen attacks of what was regarded clinically as diphtheria the organism was non-toxigenic. These cases have been grouped together and will be considered separately. Of the remaining sixty-two patients, three had a history of a previous attack of the disease, thirty-five had had a full course of prophylactic inoculation (0·2 and 0·5 ml. A.P.T. at intervals of 4 weeks or three 1 ml. doses of T.A.F. at intervals of 3 weeks), seven had had an incomplete course of inoculation and seventeen had not been inoculated. Those previously immunized had been inoculated between 1 month and 10 years before developing diphtheria. Only one patient had been inoculated less than 5 months previously, but six patients had been inoculated less than 1 year previously and twenty-two within 4 years. Each of the seven incompletely inoculated persons had had one dose of diphtheria prophylactic previously; one patient developed the disease before immunization could be completed and the rest did not attend for a second injection. Patients inoculated within the previous 4 years had received A.P.T.; those inoculated earlier had had T.A.F. or one of the older types of prophylactic.

The ages of the patients were between 3 and 24 years. The average age of the fully inoculated group was 10·9 years and that of the control non-inoculated group 11·9 years. In the control group there were ten males and seven females, while in the inoculated group there were twenty-four females and eleven males. The preponderance of females in the latter group is possibly significant, as a higher incidence of the disease in females has been noted elsewhere (Walker, 1947), especially among adults (Macdonald, 1946; Mortensen, 1946).

The membrane was situated on the fauces in all cases except two, namely, one case of laryngeal diphtheria in the non-inoculated group and one case of nasal diphtheria in the incompletely inoculated group. The nose was also affected in one patient in the inoculated group and one in the control group. No patient died.

Distribution of types of Corynebacterium diphtheriae

The routine typing of *C. diphtheriae* isolated from cases occurring in Cardiff and the neighbouring part of the county of Glamorgan was started in 1939. *Gravis* type infections were not noted until early in 1940. In 1941 only 6 % of the 1704 strains isolated were *gravis* and in 1942 only 7 % of 1512 strains (*Monthly Bulletin*, 1945). There was, however, an increase in the incidence of *gravis* during the period of this investigation; in 1943, 21 % of 1301 infections were due to *gravis* and in 1944, 31 % of 1185 infections. *Intermedius* was throughout the predominant type. In Table 1 the distribution of types in the inoculated and control groups is compared with that in all cases diagnosed in Cardiff and the neighbouring part of the county of Glamorgan during 1943 and 1944. In the control group *mitis* infections were commonest. The distribution of types in this small group of non-

Table 1. *Types and serological groups of strains of* Corynebacterium diphtheriae *isolated, compared with their incidence in the surrounding district*

Source of strains	*Intermedius* type			*Mitis* type								*Gravis* type				
	Serological group	Total strains		Serological group						Total strains		Serological group			Total strains	
	1	No.	%	1	4	7	10	Inagglutinable*	Not grouped†	No.	%	1	Inagglutinable‡	Not grouped†	No.	%
From fully inoculated patients	24	24	68·6	5	0	0	1	3	0	9	25·7	2	0	0	2	5·7
From non-inoculated patients	6	6	35·3	1	0	0	1	3	0	9	52·9	1	1	0	2	11·8
Non-toxigenic strains	2	2	10·5	2	1	2	0	4	1	10	52·6	4	2	1	7	36·9
Total strains isolated from cases of diphtheria in Cardiff and part of Glamorgan in 1943 and 1944§	—	916	39·0	—	—	—	—	—	—	789	33·6	—	—	—	624	26·6

* Mostly strains which did not ferment starch and gave colonies intermediate in characters between *gravis* and *mitis*.
† Strains not submitted for grouping.
‡ Mostly gave atypical colonies.
§ There were in addition seventeen strains which were not classified.

immunized persons is thus different from that found in all cases diagnosed and may have been due to a selection of mild cases. In the inoculated group two-thirds of the infections were due to *intermedius*. The preponderance of *intermedius* infections was considerably greater than was found in all cases diagnosed over that period. All the severe infections in the inoculated group were due to *intermedius*. In the non-inoculated each of the three types gave rise to severe cases, and in the small number of cases investigated there did not appear to be any difference in severity between *mitis* and *intermedius* infections. The two *gravis* infections in this group were both severe.

Serological grouping showed some of the strains of *mitis* to belong to groups 1 and 10; others with atypical colonies were inagglutinable. Three strains of *gravis* were identified as group 1, a fourth strain being atypical and inagglutinable. All the strains of *intermedius* were agglutinated by a group 1 antiserum.

Level of antitoxin in the blood on admission

The level of antitoxin in the serum was estimated from blood samples taken immediately each patient was admitted to hospital (Table 2). Schick regarded

Table 2. *Results of estimations of antitoxin in sera on admission*

Antitoxin in serum on admission (units/ml.)	No. of cases			
	Not immunized (17)	Fully inoculated (35)	Incompletely inoculated (7)	Previous history of diphtheria (3)
1·0	0	0	1	0
0·4	1	0	0	0
0·2	0	1	0	0
0·05	0	4	0	0
0·02	1	8	1	0
0·01	0	5	0	1
0·005	0	3	0	0
0·002	0	2	0	1
0·001	2	5	0	0
<0·001	13	7	5	1

0·03 unit/ml. or more of antitoxin in the serum as adequate for protection. Some British and other authorities have considered persons with as little as 0·01 unit/ml. as adequately protected. Among thirty-five fully inoculated patients, seventeen (48·6%) had less than 0·01 unit/ml. but only five (14·3%) had amounts of antitoxin greater than 0·03 unit/ml. In four patients the level of antitoxin was only slightly higher, namely, 0·05 unit/ml. The remaining patient had 0·2 unit/ml., but this sample of blood had been taken on the 6th day of the disease. It has been shown that in persons who have been previously inoculated and have thus been conditioned to react to the antigenic stimulus of diphtheria toxin, a rapid rise in the level of antitoxin occurs about the 7th to 12th day of the disease (Hartley *et al.* 1950). Clinicians have noted that in the inoculated person the onset of diphtheria is often insidious, and that it may be difficult to determine the actual day on which the disease started; the disease may have thus lasted longer than the history would indicate. It is therefore possible that the high level of antitoxin in

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this one patient may have been due to the blood sample having been taken after there had been an increase in antitoxin.

Owing to delays in making the diagnosis of diphtheria the blood samples in the whole series were taken at varying times after the onset of the disease between the 1st and 6th days. Comparison of the results of antitoxin estimations made on the different days of the disease did not suggest that, with the possible exception already mentioned, the results were affected by the day of the disease on which the sample was taken. Ipsen (1946) came to a similar conclusion.

Of the seventeen non-immunized patients thirteen had no detectable antitoxin, two had 0·001 unit/ml., one had 0·02 unit/ml. and one had 0·4 unit/ml. Five of the seven incompletely inoculated patients had no measurable antitoxin, one had 0·02 unit/ml. and one had 1 unit/ml. The three patients who had had previous attacks of diphtheria all had amounts of antitoxin below that regarded as conferring protection.

Among the incompletely inoculated and non-inoculated patients two with moderately severe attacks of diphtheria had 1 and 0·4 unit/ml. respectively of antitoxin in their blood. The samples of blood were withdrawn on the 3rd and 2nd days of the disease. If it is presumed, because of the precautions taken, that the samples were not contaminated with the antitoxin used therapeutically, it is of interest that these patients had moderately severe attacks of diphtheria, although at the beginning of the disease their blood contained amounts of antitoxin considerably greater than that usually regarded as capable of conferring protection. Hartley *et al.* (1950) encountered many similar examples.

Severity of the disease

In the fully inoculated group nine patients were regarded as suffering from severe attacks of diphtheria, ten from moderately severe and sixteen from mild attacks. When the whole group was divided into those with 0·01 unit/ml. or more of antitoxin in their blood on admission and those with less, there was no apparent difference in the severity of the disease in the two groups. Even those patients with more than 0·03 unit/ml. did not have less severe attacks. Ipsen (1946) noted a similar lack of correlation between the antitoxin level in the serum and the severity of the disease, but Hartley *et al.* (1950) had the opposite experience. Among the non-immunized patients there were nine severe attacks, five moderately severe and three mild. This group is too small and unrepresentative for the severity of the disease in the inoculated and the non-inoculated to be compared.

Level of antitoxin in the blood in convalescence

Antitoxin estimations were made on samples of blood obtained from twenty-three fully inoculated patients during convalescence (Table 3). Two sera had 2 units/ml.; with one exception the rest had more, ten sera having more than 20 units/ml. The serum from one patient who was an exception contained 0·02 unit/ml. on admission and 0·005 unit/ml. 11 weeks later. The patient, who had had a mild attack of diphtheria, had apparently failed to react adequately to the antigenic stimulus of what was shown to be a toxigenic strain of *C. diph-*

theriae. The levels of antitoxin reached in convalescence by those with 0·01 unit/ml. or more in their sera on admission were not significantly different from the levels attained by those with less on admission. Even those patients with no detectable antitoxin on admission had an average of about 9 units/ml. in convalescence, compared with about 12 units/ml. for the whole group. There was no significant difference between the increases in antitoxin in those with severe infections and in those with mild infections.

Estimations of antitoxin in convalescence on seventeen patients, not previously immunized and with no previous history of diphtheria, showed increases above the level on admission, which were markedly less than in the immunized patients (Table 3). Two patients had as little as 0·002 unit/ml., and no patient had more than 4 units/ml., whereas eighteen of the twenty-three immunized patients (78·3 %) had more than 5 units/ml. The four patients, who on admission had measurable amounts in their sera, in convalescence had levels of antitoxin rather

Table 3. *Amounts of antitoxin in the sera of twenty-three fully inoculated patients and seventeen non-immunized patients during convalescence*

	No. of cases	
Units per ml.	Fully inoculated	Non-immunized
20–25	10	0
15–20	1	0
10–15	4	0
5–10	3	0
4	2	2
2	2	3
0·4	0	1
0·2	0	3
0·1	0	3
0·02	0	1
0·01	0	2
0·005	1	0
0·002	0	2

higher than the average; one serum contained 4 units/ml., two contained 2 units/ml. and one 0·1 unit/ml.

Antitoxin estimations on sera obtained in convalescence from five patients who had had only one dose of diphtheria prophylactic showed amounts of 40, 10, 4, 0·4 and 0·2 units/ml. The three patients with a previous history of diphtheria had 10, 4 and 2 units/ml. antitoxin in their sera after recovery.

Clinical diphtheria associated with non-toxigenic strains of Corynebacterium diphtheriae

The strains of *C. diphtheriae* isolated from nineteen infections (23·5 %) involving eighteen patients among the total examined were found to be avirulent for guinea-pigs (Table 4). One patient was admitted twice during the investigation at an interval of 3 months; on each occasion a clinical diagnosis of diphtheria was made, yet the organisms isolated were non-toxigenic.

The affected patients consisted of ten females and eight males, aged between

4 and 23 years, with an average of about 12 years (Table 5). Two cases were regarded as nasal diphtheria and the rest as faucial. In the assessment of severity one was regarded as severe, eight as moderately severe and ten as mild. Thus, although the disease was much milder than in those patients from whom toxigenic organisms were isolated, nearly half were regarded as suffering from at least a moderately severe attack of diphtheria. All except one were given antitoxin in doses between 1000 and 40,000 units.

Ten of the nineteen strains of *C. diphtheriae* were *mitis* (Table 1), two being identified serologically as group 1, one as group 4 and two as group 7. Four strains could not be grouped serologically and gave atypical colonies, but similar strains were met with which were virulent. There were two strains of *intermedius*, group 1. An additional two non-toxigenic group 1 strains were isolated from

Table 4. *Clinical cases of diphtheria from whom non-toxigenic diphtheria bacilli only were isolated*

Type of case	Fully inoculated	Incompletely inoculated	Previous history of diphtheria	Non-immunized	Total cases
No. of cases with toxigenic organisms	35	7	3	17	62
No. of cases with non-toxigenic organisms	9	3	4	2*	19
Percentage of cases with non-toxigenic organisms	20·5	30	57·1	10·5	23·5

* One patient had two separate attacks.

carriers. Seven of the strains were *gravis*, four being identified as group 1. Although the colonial appearance of these strains appeared typical on primary isolation, Prof. J. W. McLeod, who reinvestigated five *gravis*, group 1, strains after they had been maintained by subculture, reported that they were atypical in morphology and in colonial appearances (McLeod & Robinson, 1948). He confirmed that they were avirulent for guinea-pigs.*

Six of the patients infected with non-toxigenic *gravis* strains had moderately severe attacks of the disease and the one other patient had a mild attack. Both *intermedius* infections were mild. There were one severe, two moderate and seven mild *mitis* infections.

Thirteen of the eighteen patients had in their sera on admission more than 0·03 unit/ml. of antitoxin (Table 5). In eight out of twelve patients, whose sera were examined for antitoxin in convalescence, the titre was not significantly raised. Three patients had an increased amount of antitoxin in convalescence, two increasing from 0·02 to 0·1 unit/ml. and one from 0·05 to 0·2 unit/ml. It is possible that the increase in antitoxin in these patients resulted from cross-infection with toxigenic diphtheria bacilli in hospital. The third patient developed rhinitis in hospital, and toxigenic diphtheria bacilli were in fact isolated from her nose.

* A sixth strain, sent to Prof. McLeod at the same time, was found to be virulent. It is not included in this report, because it was isolated from a patient who was finally diagnosed as a carrier.

Table 5. *Details regarding patients from whom only non-toxigenic strains of* Corynebacterium diphtheria *were isolated*

Sex	Age	Previous history	Site of membrane	Severity	Units of anti-toxin adminis-tered	Strain of *C. diphtheriae*		Antitoxin on admission		Antitoxin in convalescence	
						Type	Serological group	Day of disease	Units/ml.	Week of disease	Units/ml.
F.	7	Immunized	Faucial	Mild	8,000	*Mitis*	1	3	1·5	—	—
F.	10	Immunized	Faucial	Moderate	40,000	*Gravis*	1	2	1·0	—	—
F.	7	Immunized	Nasal	Mild	8,000	*Intermedius*	1	3	1·0	17	0·2
M.	13	Immunized	Faucial	Moderate	30,000	*Gravis*	1	5	1·0	—	—
F.	20	Immunized	Faucial	Moderate	10,000	*Gravis*	Inagglut.	4	1·0	—	—
F.	13	Immunized	Faucial	Moderate	20,000	*Mitis*	Inagglut.	2	0·8	6	0·4
M.	15	Immunized	Faucial	Mild	8,000	*Gravis*	Not grouped	3	0·02	8	0·1
M.	4	Immunized	Faucial	Mild	8,000	*Mitis*	7	2	0·005	13	0·01
M.	16	Immunized	Faucial	Moderate	20,000	*Gravis*	Inagglut.	3	0·005	6	0·01
F.	13	Incompletely immunized	Faucial	Very mild	10,000	*Mitis*	1	3	0·2	7	0·2
M.	23	Incompletely immunized	Faucial	Mild	10,000	Atypical *mitis*	Inagglut.	5	0·02	6	0·1
M.	10	Incompletely immunized	Faucial	Mild	1,000	Atypical *mitis*	Inagglut.	9	< 0·001	—	—
F.	9	Diphtheria 3 years previously	Faucial	Mild	10,000	*Intermedius*	1	4	10	8	8
F.	15	Diphtheria 1 year previously	Faucial	Mild	10,000	Atypical *mitis*	Inagglut.	2	2	—	—
M.	13	Diphtheria 8 years previously	Faucial	Moderate	40,000	*Gravis*	1	4	0·2	6	0·4
F.	17	Diphtheria 14 years previously	Faucial	Moderate	None	*Mitis*	7	7	0·05	8	0·2*
M.	6	Not immunized	Nasal	Mild	10,000	*Mitis*	Not grouped	3	0·8	5	1·0
F.	17	Not immunized	Faucial	Moderate	20,000	*Gravis*	1	2	0·2	5	0·2
		Second attack 3 months later	Faucial	Severe	40,000	*Mitis*	4	2	0·4	7	0·4

* Developed in hospital a nasal discharge containing virulent *C. diphtheriae*.

A third sample of blood taken 11 weeks after admission showed that the amount of antitoxin had increased further to 0·8 unit/ml. In one patient the amount of antitoxin decreased from 1 unit/ml. on admission to 0·2 unit/ml. after recovery.

It appeared important to determine whether any other organism could be incriminated as the cause of these infections. In the course of the routine bacteriological investigation a profuse growth of *Streptococcus pyogenes* was obtained from the throat of one patient, and a moderate growth from another. Small numbers of *Str. pyogenes* were isolated from five others. Vincent's organisms in small numbers were found in the throat of one patient. *Str. pyogenes* and Vincent's organisms were demonstrated in an approximately similar proportion of patients from whom virulent diphtheria bacilli were isolated.

In the patients in this group diagnosed as suffering from faucial diphtheria the onset of the disease was sudden. All complained of sore throat, and headache was common; a few vomited. Although the majority were apyrexial on admission a few had temperatures up to 101° F., which usually fell to normal within 24 hr. All the patients had one or more patches of whitish exudate on the tonsils, which were usually enlarged. In most there was a slight or moderate enlargement of one or both tonsillar lymph nodes. A 'diphtheritic odour' was detected in two patients. There was a purulent nasal discharge of sudden onset in the two patients in whom nasal diphtheria was diagnosed. No patient suffered from carditis or paralysis as a complication.

One patient was admitted twice during the investigation. She was a girl, aged 17 years; 3 years previously she had been Schick-negative, but had never been immunized. She was admitted first on 22 July 1943 with a diagnosis of faucial diphtheria. The illness had started suddenly the previous day with sore throat and headache. On admission her temperature was 101° F., but fell to normal next day; the pulse rate was 124 and respiration rate 22. The tonsils were enlarged, and there was a moderate-sized patch of exudate on the right tonsil and a small patch on the left. The fauces were injected and the tonsillar lymph nodes were enlarged. She was regarded as suffering from a moderately severe attack of diphtheria and was given 30,000 units of antitoxin. There was a faint trace of albumin in the urine during the first 3 days. *C. diphtheriae, gravis* type, was grown from her throat, shown to be avirulent for a guinea-pig and identified serologically as group 1. Other organisms of pathogenic significance were not found. On admission her serum contained 0·2 unit/ml. of antitoxin, and the same amount was present 5 weeks later. She made an uneventful recovery and was discharged after 6 weeks.

On 10 October 1943 she was readmitted again with a diagnosis of faucial diphtheria. She had been taken ill the previous day with a sore throat, and on admission her temperature was 100° F., pulse 120, respiration 22. The temperature gradually fell to normal by the 3rd day. When examined a large patch of greyish white exudate was found on the right tonsil and a medium-sized patch on the left. The cervical lymph nodes were not enlarged. A 'diphtheritic odour' was noted. A severe attack of diphtheria was diagnosed and she was given 40,000 units of antitoxin. *C. diphtheriae, mitis* type, serological group 4, avirulent for a guinea-

pig, was grown from her throat together with a few colonies of *Str. pyogenes*. Samples of serum obtained on admission and again 7 weeks later contained 0·4 unit/ml. of antitoxin. She was discharged after 6 weeks.

DISCUSSION

A surprising observation made during this inquiry was that from 23·5 % out of a total of eighty-one patients diagnosed as suffering from diphtheria non-toxigenic diphtheria bacilli only were isolated. In a small group of seven patients regarded as suffering from a second attack of diphtheria the organism isolated from four was non-toxigenic. From one patient admitted twice, with a diagnosis of diphtheria on each occasion, the organisms obtained by culture (*gravis* and *mitis* respectively) were both non-toxigenic.

It is usual to regard the clinical manifestations of diphtheria as resulting from the action of the specific exotoxin; non-toxigenic strains of *C. diphtheriae* are accepted as non-pathogenic and unable to cause diphtheria in the human being. As an explanation of our findings it might be suggested that both toxigenic and non-toxigenic diphtheria bacilli were present in the upper respiratory tract, but that only a colony of the latter was picked off for further study. Alternatively, the virulence tests may have been at fault, or an organism, although unable to kill a guinea-pig, may have been able to form toxin inside the human body. There is, however, corroborative evidence that these patients were not exposed to the action of exotoxin. Estimations of antitoxin in the blood showed the majority to have at the onset of the disease amounts of antitoxin usually regarded as more than adequate for protection. Moreover, when a second sample of blood was examined some weeks later it was found that there had been no increase in antitoxin. In patients infected by toxigenic diphtheria bacilli examination of the blood in convalescence showed an increase in antitoxin, which was considerable in patients who had been previously inoculated. Virulence tests on a number of the strains carried out independently by four other laboratories using different techniques confirmed our results. Mr L. M. Holt, M.Sc., The Wright-Fleming Institute, St Mary's Hospital, London, also kindly tested by a sensitive method three representative strains for ability to form exotoxin and found them completely non-toxigenic.

The evidence suggests that none of the eighteen patients was infected by toxigenic diphtheria bacilli, and yet all had attacks of a disease regarded by an experienced clinician as diphtheria. The infection was usually mild, although one case was regarded as severe and eight as moderately severe. No conclusion can be drawn regarding the aetiology of the disease. Although no other recognized pathogen was isolated by culture from the majority of the patients, it is possible that some unidentified bacterium or virus was responsible. A type of exudative tonsillitis and pharyngitis, probably due to a virus, has been described, but the lesions more closely resembled those of a haemolytic streptococcal infection (Commission, 1947). Alternatively, the non-toxigenic strains of *C. diphtheriae* themselves, either alone or in conjunction with another organism, may have caused the disease.

The very potency of the diphtheria exotoxin has focused attention on it, but it may be that other factors contribute to the virulence of *C. diphtheriae*, particularly to its local invasive action. A report by Frobisher, Parsons & Updyke (1947) tends to confirm our findings. Without giving figures they stated that in Baltimore during 1945–6, and in investigations made elsewhere in the U.S.A. and in other countries, there had been an increasing number of instances where only avirulent organisms could be isolated from cases of clinical diphtheria. They reviewed the evidence collected by Frobisher & Parsons (1940, 1943) showing that diphtheria bacilli, both toxigenic and non-toxigenic, contained an endotoxin, lethal and neurotropic for mice when inoculated intracerebrally. They concluded that the ordinary virulence tests for ability to produce exotoxin did not necessarily reveal all the pathogenic potentialities of an organism for the human being, and that non-toxigenic strains may by some unknown mechanism produce a diphtheria-like disease. O'Meara (1940) also believed that virulent diphtheria bacilli contained factors, apart from the exotoxin, which determined virulence. T'ung (1945) showed that non-toxigenic diphtheria bacilli could produce a pseudomembranous inflammation of the chorio-allantoic membrane of the developing chick embryo. In the series of cases reported here the local lesions, although in no case extensive and always limited to the tonsillar area, were not distinguishable from those of typical faucial diphtheria; infection of the nose resulted in a purulent discharge. General toxaemia was usually mild and no patient developed paralysis or carditis.

It is not the usual practice to perform a virulence test on an organism isolated from an acute case of diphtheria when the diagnosis is not in doubt clinically. McLeod (1943) collected the results of a large number of virulence tests carried out by different workers in several countries; these showed that 98 % of *gravis* strains and 96 % of *intermedius* were virulent. He concluded that virulence tests on typical strains of *gravis* and *intermedius* were superfluous. In the investigations reported here seventeen strains of *gravis*, serological group 1, were encountered and eleven (64·7 %) were non-toxigenic. There were also four non-toxigenic strains of *intermedius* out of thirty-nine strains examined (10·3 %). These results, together with those of Frobisher *et al.*, and an earlier report by Jordan, Smith & Kingsbury (1922), who, in investigating strains of *C. diphtheriae*, isolated several non-toxigenic strains from clinical cases of diphtheria, suggest that it is not always safe to assume that all strains isolated from acute cases of diphtheria are toxigenic. Observations are needed in other areas, especially where *gravis* infections predominate, to confirm and investigate further the association of non-toxigenic bacilli with clinical diphtheria.

In the final analysis of the results to obtain a comparison between those patients who had been previously inoculated against diphtheria and those who had not, only patients were included from whom virulent diphtheria bacilli were isolated. It seemed justifiable to exclude patients from whom non-toxigenic bacilli only were isolated and to consider them separately. The aetiology of such infections has not been established, and though clinically they appeared to be diphtheria they must for the moment be regarded as distinct from a disease now believed to

be associated with the action of a specific exotoxin. Moreover, artificial immunization is effected using modified exotoxin, and the immunological state is assessed by estimating antitoxin levels.

There have been numerous reports of diphtheria occurring in immunized persons; in this country the majority of such infections have been due to *gravis* strains (Dudley, May & O'Flynn, 1934; Underwood, 1935; Parish & Wright, 1935; Hartley *et al.* 1950). In Manchester, where *intermedius* infections were ten times as common as *gravis*, there were eight *gravis* infections to four *intermedius* in inoculated persons (Robinson & Marshall, 1934). The suggestion has been made that diphtheria occurs in inoculated persons owing to a particularly virulent strain of *gravis* breaking down the immunity. The results obtained in Cardiff are therefore of particular interest. In this area *intermedius* infections predominated and caused about 39% of the total diphtheria cases; the incidence of *gravis* infections progressively increased during the 2 years over which the inquiry extended, but did not exceed 32%. Among the inoculated persons more than two-thirds of the infections were due to *intermedius* and only 5·7% to *gravis*. Thus in Cardiff, where *gravis* was not of high virulence, the *intermedius* type proved to be the most likely to overcome partial immunity.

Whereas it would appear that diphtheria in the inoculated does not occur because of the peculiar virulence of the infecting organism, there is evidence suggesting that it usually affects those with less antitoxin than that regarded as necessary for their protection. On admission to hospital sera from only five out of thirty-five fully inoculated patients contained more than 0·03 unit/ml. of antitoxin. In four the amount was only slightly in excess (0·05 unit/ml.). The fifth patient had 0·2 unit/ml., but the sample had been taken on the 6th day of the disease at the earliest; it is possible that antitoxin had already increased in the blood above the level present at the onset. It is often believed that persons with 0·03 unit/ml. of antitoxin, or even 0·01 unit/ml., are immune, but, as Ipsen (1946) pointed out, it is probable that there is no threshold value giving *absolute* immunity. Rather it is true that the higher the level of antitoxin is above 0·01 unit/ml. the better the protection afforded. The antitoxin estimations on our cases showed the level to be either below that necessary for immunity or in its neighbourhood.

There would have been a different picture if cases from whom only non-toxigenic diphtheria bacilli were isolated had been included with the others in the group. Then out of a total of forty-four patients there would have been eleven with more than 0·03 unit/ml. of antitoxin, including one with 1·5 units/ml. and four with 1·0 unit/ml. The latter five patients were all infected with non-toxigenic bacilli. This emphasizes the need for routine virulence tests in this type of investigation. If non-toxigenic bacilli can be pathogenic for the human being by virtue of some component other than the exotoxin, resistance to such an infection is obviously largely independent of antitoxin. Previous investigations (Underwood, 1935; Ipsen, 1946; Hartley *et al.* 1950) have revealed instances of diphtheria occurring in persons with considerable amounts of circulating antitoxin. Also in this series one incompletely inoculated person had 1 unit/ml. of antitoxin in his serum on the 3rd day of an attack of diphtheria and one non-inoculated person similarly

had 0·4 unit/ml. on the 2nd day. Excluding the possibility of false results due to contamination of blood samples with antitoxin used therapeutically or collection of the first blood sample at too late a stage in the disease, some of the earlier observations may have been due to inclusion of cases infected by non-toxigenic organisms. However, if non-toxigenic bacilli can produce a disease resembling diphtheria due to a factor other than exotoxin, it is probable that toxigenic strains can also have the same effect, independent of the action of exotoxin which will not be prevented by an adequate level of antitoxin. If this hypothesis is correct, it would not be surprising if diphtheria-like infections associated with virulent diphtheria bacilli were to occur occasionally in persons with adequate amounts of circulating antitoxin.

Estimations of antitoxin in the blood were again made after recovery from the disease. These samples were taken at least 5 weeks after admission to hospital, when it was presumed that the antitoxin administered therapeutically had fallen to a low level. Only three of the forty-eight convalescent sera examined contained less than 0·01 unit/ml. The amount of antitoxin was considerably greater in those patients who had been inoculated previously. Sera from fifteen out of twenty-three fully inoculated patients (65·2 %) contained more than 10 units/ml., whereas none of the non-immunized had more than 4 units/ml. Even patients who had only had one dose of diphtheria prophylactic had high levels of antitoxin in convalescence. Possibly in some of the samples a proportion of the antitoxin estimated was that which had been administered therapeutically. This error, however, would be likely to be the same in each of the groups. The difference in the findings between the immunized and the non-immunized is so marked as to be highly significant. It would appear that persons, previously exposed to the antigenic stimulus of diphtheria toxin, react during an attack of diphtheria, even though there was little or no antitoxin in their blood at the onset, by the formation of considerably more antitoxin than was formed by those not previously exposed. One of the inoculated patients failed to react adequately; the level of antitoxin after recovery, 0·005 unit/ml., could not be considered adequate to ensure protection against a second attack.

SUMMARY

A series of thirty-five patients suffering from diphtheria, who had been previously fully inoculated against the disease and from whom toxigenic diphtheria bacilli were isolated, were investigated and compared with seventeen patients who had not been inoculated. There were smaller groups of those patients who had had incomplete courses of immunization and of those who had had previous attacks of the disease.

Two-thirds of the infections in the inoculated were due to *Corynebacterium diphtheriae*, *intermedius* type. *Intermedius* infections were proportionately more numerous in the inoculated than in the uninoculated, whereas *gravis* infections were less frequent.

Estimations of the antitoxin in the blood were made on admission. The inocu-

lated patients had levels of antitoxin below, or in the neighbourhood of, that believed to confer protection.

In the inoculated group severity did not appear to be influenced by the level of antitoxin in the blood on admission.

Antitoxin in the blood was found to have increased after recovery from the disease in all except one patient; the increase was considerable in those who had been inoculated previously.

From 23·5% of all cases of clinical diphtheria investigated only non-toxigenic diphtheria bacilli (*gravis*, *intermedius* and *mitis* types) were isolated. These cases were considered separately. On admission the majority had in their blood considerable amounts of antitoxin, which did not increase after recovery. No other pathogenic organisms were regularly found. The possibility is discussed that under certain conditions non-toxigenic *C. diphtheriae* may be able to cause a diphtheria-like disease.

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REFERENCES

Commission on Acute Respiratory Diseases (1947). *J. Amer. med. Ass.* **133**, 588.
Dudley, S. F., May, P. M. & O'Flynn, J. A. (1934). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 195.
Frobisher, M., Jr. & Parsons, E. I. (1940). *Proc. Soc. exp. Biol., N.Y.*, **45**, 165.
Frobisher, M., Jr. & Parsons, E. I. (1943). *Amer. J. Hyg.* **37**, 53.
Frobisher, M., Jr., Parsons, E. I. & Updyke, R. (1947). *Amer. J. publ. Hlth*, **37**, 543.
Hartley, P., Tulloch, W. J., Anderson, M., Davidson, W. A., Grant, J., Jamieson, W. M., Neubauer, C., Norton, R. & Robertson, G. H. (1950). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 272.
Ipsen, J. (1946). *J. Immunol.* **54**, 325.
Jordan, J. H., Smith, F. & Kingsbury, A. N. (1922). *Lancet*, **2**, 1052.
Macdonald, H. R. (1946). *Canad. J. publ. Hlth*, **37**, 391.
McLeod, J. W. (1943). *Bact. Rev.* **7**, 1.
McLeod, J. W. & Robinson, D. T. (1948). *Lancet*, **1**, 97.
Mon. Bull. Min. Hlth and E.P.H.L.S. (Med. Res. Coun.) (1945). **4**, 152.
Mortensen, V. (1946). *Acta med. scand.* **125**, 283.
O'Meara, R. A. Q. (1940). *J. Path. Bact.* **51**, 317.
Parish, H. J. & Wright, J. (1935). *Lancet*, **1**, 600.
Robinson, D. T. & Marshall, F. N. (1934). *J. Path. Bact.* **38**, 73.
T'ung, T. (1945). *Amer. J. Hyg.* **41**, 57.
Underwood, E. A. (1935). *Lancet*, **1**, 364.
Walker, J. V. (1947). *Lancet*, **1**, 422.

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