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An outbreak of streptococcal sore throat and rheumatic fever in a Royal Air Force Training camp; significance of serum antibody to M-associated protein

By JEAN P. WIDDOWSON, W. R. MAXTED,
Cross-Infection Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT
C. W. NEWRICK and D. PARKIN
R.A.F. Institute of Pathology & Tropical Medicine, Halton, Aylesbury, Bucks

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SUMMARY

A large outbreak of streptococcal sore throat in a Royal Air Force Training Camp resulted in five cases of rheumatic fever among the 16- to 18-year-old apprentices, and one case in a 33-year-old airman. The most prevalent type of group A streptococcus isolated from throat swabs was M-type 5 and there was serological evidence that at least four of the rheumatic fever (R.F.) cases were due to this type.

Among the patients with uncomplicated throat infection the anti-streptolysin O (ASO) and anti-deoxyribonuclease B (anti-DNAase B) responses were in general rather low, even where there was evidence of protective antibody against type 5. However, a combination of the results of the ASO and anti-DNAase B tests gave an estimate of the extent of streptococcal infection 15–25% higher than did either test alone.

The titres of antibody to M-associated protein (MAP) were $\geqslant 60$ in all the R.F. patients, and in about 50% of the other patients with ASO titres $\geqslant 200$. This figure is unusually high compared with data from several other outbreaks of streptococcal infection due to different serotypes and also greatly exceeds comparable figures for cases of sporadic sore throat and acute glomerulonephritis.

INTRODUCTION

In the spring of 1970 a large outbreak of streptococcal sore throat occurred among the apprentices in the Royal Air Force Training Camp at Halton, Bucks. Over 400 of the 1750 youths aged 16–18 years suffered from streptococcal sore throat and five of them had attacks of rheumatic fever. In the latter part of the outbreak, and subsequently, we took advantage of this situation to study the antibody response in rheumatic fever patients and to compare it with the response in other apprentices who escaped this complication.

MATERIALS AND METHODS

Diagnosis and treatment of streptococcal infection

Before 16 April 1970 the practice had been to examine clinically all apprentices reporting to the Station Sick Quarters with sore throat; if there was objective evidence of acute tonsillitis, the patient was admitted, a throat swab was collected, and penicillin treatment was begun; if not, he was given an analgesic and an antiseptic gargle, but was admitted only if the clinical condition warranted this. Routine penicillin treatment comprised phenoxymethyl penicillin 125 mg, 4 times a day for 7 days. From 16 April to 21 May all patients complaining of sore throat were treated with penicillin and a throat swab examined from each.

Throat swabs were cultured on horse-blood agar and representative β-haemolytic colonies were examined. Before 16 April these were screened for bacitracin sensitivity (Maxted, 1953) but after this date they were grouped serologically (Maxted, 1948) and a selection of the group A streptococci were sent to Colindale for typing.

Streptococcal typing

Streptococci were typed by T-agglutination (Griffith, 1934) and the M-precipitin method (Swift, Wilson & Lancefield, 1943). Rabbit antisera were prepared by the Streptococcus Reference Laboratory, Colindale.

Human sera

Serial samples of serum from patients with rheumatic fever and single samples from 45 cases of sore throat and 91 symptomless apprentices were tested for anti-streptolysin O, anti-DNAase B, anti-MAP, and type-specific antibodies against M-types 5, 18 and 58.

Anti-streptolysin O. ASO titres were determined by a spectrophotometric method based on that of Gooder & Williams (1961) and Gooder (1961).

Anti-deoxyribonuclease B. Anti-DNAase B titres were determined by the micro-method of Nelson, Ayoub & Wannamaker (1968).

Antibody to M-associated protein. Anti-MAP titres were determined by a complement fixation test (CFT) with the purified M-protein of a type 30 strain (Widdowson, Maxted & Pinney, 1971). The use of a type 30 M-protein virtually rules out the possibility of fixation of complement by a patient's serum due to the presence of type-specific antibodies, since type-30 infections are extremely rare in Britain. The CFT titre of a serum was therefore taken as a measure of antibody to the non-specific part of the M-protein complex.

The bactericidal test. This test for M-antibody was done as described by Maxted, Widdowson & Fraser (1973). All sera were tested for the presence of type-specific M-antibody to the three types of streptococci prevalent in the camp during the outbreak. These were M-types 5, 18 and 58. The sera were first treated with penicillinase (Burroughs Wellcome & Co. Ltd.) to destroy any penicillin present. Antibody to the type under test was considered to be present if $++++$ growth in the control was reduced to $-$, $+$, or $++$ growth in the test. Sera which

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showed only slight depression of growth (+ + +) were retested using 0·04 ml of serum. If the depression of growth was then increased, the serum was considered to contain antibody. None of the sera tested showed inhibition of the growth of all three types, which ruled out the possibility of killing by a non-specific mechanism, e.g. presence of residual antibiotics in the serum.

RESULTS

History of the outbreak

The frequency of sore throat began to increase late in March, and by the second week in April had risen from the usual level of about 3 cases a day to 15 cases a day. Up to 16 April about 150 of the apprentices had reported sick with sore throat. Three cases of rheumatic fever were diagnosed between 8 and 10 April and at about the same time a fourth case, a 33-year airman from a neighbouring unit whose only previous contact with Halton had been attendance at the Ear, Nose and Throat Out-patient Clinic, was admitted to the Sick Quarters with the disease.

Between 16 April and 21 May a further 270 cases of sore throat were reported and group A streptococci were isolated from over half of them. Two more cases of rheumatic fever occurred on 21 and 24 April respectively. A random selection of 34 group A streptococci isolated from patients with tonsillitis during this period were typed. Blood samples were collected from 92 of the apprentices on or about 25 April, about 4 weeks after the beginning of the outbreak.

On 21 May the apprentices went on Whitsun leave and when they reassembled at the beginning of June the outbreak had subsided. At this time, random throat swabs were collected from a sample of the boys and 60 group A streptococcal strains were typed; samples of serum were collected from 44 boys who had not reported sick with sore throat during the outbreak, and who had negative throat swabs.

Throat swabs had not been examined for four of the rheumatic fever patients during the initial respiratory infections; bacitracin-sensitive β-haemolytic streptococci were isolated from the remaining two cases, but were not typed.

Types of streptococci isolated

The outbreak of sore throat lasted about 10 weeks, but information about the types of group A streptococci prevalent during the first four weeks is lacking. M-type 5 predominated in the sample of strains isolated in weeks 5 and 6 of the outbreak, accounting for over 50 % of the total, but smaller numbers of M-types 18 and 58 were also present (Table 1). In early June when the outbreak had subsided, type 5 was still the most prevalent, but the other two types had disappeared.

Table 1. *Types of streptococci isolated from throat swabs during the period 22 April to 8 June 1970*

Date	Type		Throat swabs	
	T	M	No. positive/total positive for group A	% positive
22 April to 7 May 1970	5/27/44	5	19/34	56
	—	18	6/34	18
	25/Imp 19	58	6/34	18
		Others	3/34	9
8 June 1970	5/27/44	5	38/60	63
	9	ND	5/60	8
		Others	17/60	28

Rheumatic fever cases; clinical histories

Patient B.M.P. aged 17 years reported sick on 4 April with a sore throat which lasted 3 days. He received no penicillin treatment and on 21 April rheumatic fever with cardiac involvement was diagnosed.

Patient T.A.G., aged 16 years, first reported sick on 13 March with swollen ankles. This persisted for about 1 week, and recurred on 27 March with a sore throat and backache. These symptoms subsided, but on 8 April he had pain in both elbows and was admitted to hospital with a diagnosis of mild rheumatic fever (no cardiac involvement) on 10 April. He had received intermittent penicillin treatment for 4 weeks before admission.

Patient A.P., aged 17 years, had tonsillitis on 13 March and received oral penicillin treatment for only 2 days. On 20 April he had a mild sore throat and was admitted to hospital on 27 April with a diagnosis of typical rheumatic fever and active carditis.

Patient P.G., aged 17 years, had a mild sore throat on 20 March which was untreated, and lasted only 48 hr. On 8–10 April he developed swollen painful joints and was admitted to hospital with a diagnosis of typical rheumatic fever, without cardiac involvement, on 13 April.

Patient C.C., aged 17 years, developed a sore throat and cough on 7 March. He received 7 days' penicillin treatment and the symptoms abated. Sore throat, fever and headache recurred on 7 April and penicillin was given for a few days. Between 10 and 20 April he developed pain and swelling in both ankles and knees and stiffness in his hands and shoulder. He was admitted to hospital late in the course of his disease, which was diagnosed as classical rheumatic fever with no evidence of cardiac damage.

Patient P.M.E., aged 33 years, developed otitis media in February and a β-haemolytic streptococcus was isolated but not typed. He had no further symptoms until 7–10 April when he developed a painful swollen left knee and a sore throat. He was admitted to hospital on 12 April with typical rheumatic fever but no evidence of carditis.

Thus, there was a history of antecedent sore throat in five of the six cases, but

Table 2. *Streptococcal antibody titres of rheumatic fever patients*

Patient number	Age (years)	Rheumatic fever	Date of bleeding	Weeks* after onset	Titre of anti-streptolysin O	Titre of anti-DNAase B	Titre of anti-MAP	Presence of type 5 M-antibody in serum taken on 13. v. 70
1	17	Typical: aortic valve damage	24. iv. 70	½	570	400	128	
			13. v. 70	3	720	1600	256	
			21. v. 70	4	520	400	256	+
			10. vi. 70	7	280	—	256	
			4. ix. 70	21	160	400	128	
2	16	Mild: no heart damage	16. iv. 70	1†	590	800	64	
			13. v. 70	4	470	800	64	
			21. v. 70	7	340	1600–3200	64	−
			10. vi. 70	10	340	1600–3200	64	
3	17	Typical: active carditis, aortic systolic murmur	29. iv. 70	1	280	800	32	
			13. v. 70	3	640	1600–3200	64	
			21. v. 70	4	590	1600	64	−
			10. vi. 70	7	300	400	64	
4	17	Typical: no heart damage	16. iv. 70	1	580	—	64	
			13. v. 70	5	560	6400	64	
			21. v. 70	6	600	—	128	+
			10. vi. 70	9	520	400	64	
5	17	Typical: no heart damage	1. v. 70	3	550	800	256	
			13. v. 70	5	630	6400	256	
			21. v. 70	6	530	3200	256	+
			10. vi. 70	9	540	400	256	
6	33	Clinically 'fairly typical' no heart damage	16. iv. 70	1	305	3200–6400	128	
			5. v. 70	4	320	—	256	
			13. v. 70	5	300	6400	128	+
			3. vi. 70	8	330	800	128	
			4. ix. 70	20	155	400	128	
		Upper limit of normal			200	250	20	

* Weeks after approximate onset of rheumatic fever symptoms.
† History indefinite; rheumatic fever may have begun 3 weeks earlier.

Table 3. *Percentages of sera with raised streptococcal antibody titres from various groups of cadets in R.A.F. Halton*

	Symptomless with negative TS*		Symptomless with positive TS†		Sore throat with negative TS†		Sore throat with positive TS†		Type 5M isolated from throat†		Rheumatic fever cases
Antibody	No.	%	No.	%	No.	%	No.	%	No.	%	
ASO titre > 200	11/44	25	17/47	34	7/22	31	6/23	26	7/16	43	6/6
Anti-DNAase B titre > 250	19/39	49	14/44	32	6/20	30	6/23	26	6/16	37	6/6
Anti-MAP titre > 20	18/44	41	26/47	55	11/22	50	10/23	43	12/16	75	6/6
Anti-M antibody to types 5, 18 or 58 present	14/44	32	20/47	40	5/22	23	8/23	31	12/16	75	4/6
Antibody to type 5 present	9/44	21	15/47	30	2/22	9	6/23	26	11/16	68	4/6
Antibody to type 18 present	5/44	12	8/47	17	2/22	9	3/23	13	1/16	6	0/6
Antibody to type 58 present	1/44	2	0/47	0	2/22	9	1/23	4	1/16	6	0/6

Negative TS = throat swab negative for β-haemolytic streptococci.
Positive TS = throat swab positive for β-haemolytic streptococci.
* Sera collected on 7 June.
† Sera collected on 25 April.

this was mild and indefinite in four cases; in two there was more than one episode of sore throat, making it impossible to determine the length of the latent period. In only one case was penicillin given for 7 days.

Antibody titres of rheumatic fever patients

The findings are summarized in Table 2. All six patients showed a moderate rise in ASO titre and a somewhat more dramatic rise in anti-DNAase B titre, which in most cases began to decline during the period of investigation. The anti-MAP titre was raised in the first serum of each series and in general showed less tendency to decline. Sera taken on 13 May from all six patients were tested in the bactericidal test and four out of six had type-specific antibody to type 5. None of the sera had M-antibodies to type 18 or 58.

Antibody titres in sera from cadets with uncomplicated respiratory infection and from symptomless controls

The data derived from ASO, anti-MAP, anti-DNAase B and type-specific antibody tests were analysed by dividing the sera into five groups. Table 3 shows the percentage of sera in each of these groups that had antibody titres above the upper limit of normal in the ASO, anti-DNAase B and anti-MAP test. The upper limit of normal for the ASO was taken as 200 (Gooder & Williams, 1961). A value of 250 was taken as the upper limit of normal for the anti-DNAase B titres. In a survey by Ayoub & Wannamaker (1962), this value was exceeded

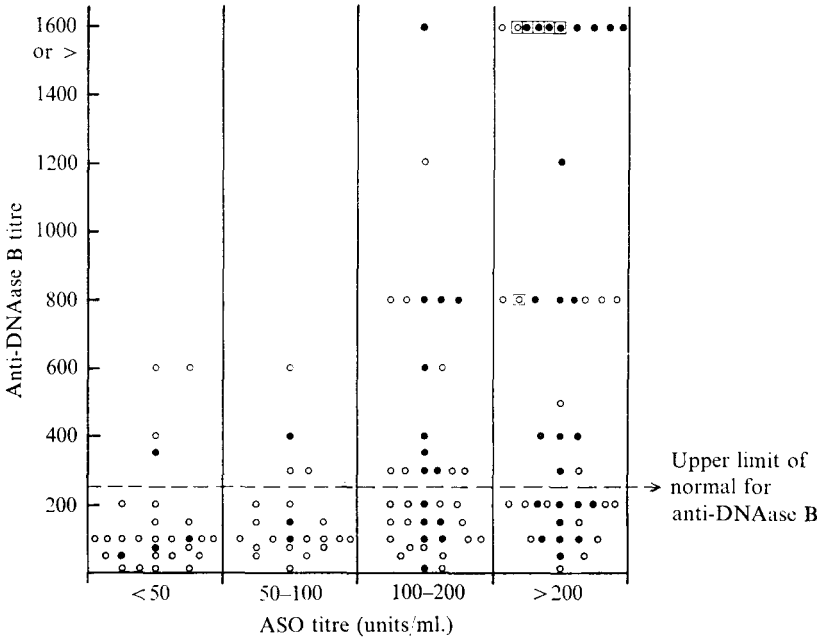

Fig. 1. The relationship of anti-DNAase B titre with ASO titre and the presence of type-specific antibodies. ●, Denotes presence of antibody to M types 5, 18 or 58. ○, denotes absence of type-specific antibody to these types. □, rheumatic fever cases.

by 15% of normal subjects tested. In similar tests in our laboratory a titre of 250 was exceeded by about 14% of persons without a history of recent streptococcal infection. In the anti-MAP test all sera with titres greater than, but not including 20, were considered to be above the upper limit of normal. About 20% of presumed normal sera examined have titres exceeding 20. Most normal individuals have titres of < 10 (Widdowson *et al.* 1971, and unpublished).

Table 3 shows the percentage of sera with evidence of protective antibody to types 5, 18 and 58. The results for the sera of the rheumatic fever patients are included for comparison, and these were derived from the antibody titres of sera taken on 13 May (see Table 2) between 3 and 8 weeks after the onset of rheumatic fever. The results for the ASO, anti-DNAase B and anti-MAP titres in Table 3 indicate very little difference between cadets with positive or negative throat swabs, or between cadets classified as symptomless and those who reported sick with sore throats, despite the difference of 6 weeks in the date of collection of the serum samples. The percentage of sera with type-specific antibody against type 5 appeared to be lower (9%) in cadets with sore throats but negative throat swabs, than in any other group, but the difference was not statistically significant at the 5% probability level ($\chi^2 = < 3{\cdot}84$).

The percentage of patients with elevated ASO and anti-DNAase B titres in the group which had type 5 M isolated from the throat did not differ significantly from the other groups in Table 3 ($\chi^2 < 3{\cdot}84$). However, the greatest difference between this group and the rest was in the number of sera with type-specific

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Table 4. *Relationship of ASO and anti-DNAase B titre with the presence of type-specific antibodies*

Anti-DNAase B titre	Sera with antibody to type 5 out of total sera with ASO titre of:			Sera with antibody to types 5, 18 or 58 out of total sera with ASO titre of:		
	< 100	100–200	> 200	< 100	100–200	> 200
> 400	0/3 (0)	4/9 (44)	11/20 (55)	0/3 (0)	5/9 (55)	12/20 (60)
250–400	1/5 (20)	3/8 (38)	3/5 (60)	2/5 (40)	4/8 (50)	4/5 (80)
< 250	2/42 (5)	3/21 (14)	7/19 (37)	5/42 (12)	6/21 (28)	9/19 (47)

Figures in parentheses represent the percentage of sera in each group with type-specific antibody.

antibody to type 5; 68% compared with an average of 21·5% for the other four groups ($\chi^2 > 6{\cdot}6$ – the difference is significant at the 1% probability level). The percentage of sera with raised anti-MAP titres (75% compared with an average of 47% for the other groups) was also significantly high ($\chi^2 > 6{\cdot}6$).

Correlation of ASO titres with anti-DNAase B titres

The anti-DNAase B titres of all the sera were plotted as a scattergram in four categories of ASO titre (Fig. 1). Although there was a general tendency for the anti-DNAase B titre to increase with the ASO titre, there were 25 of 134 sera with anti-DNAase B titres > 250 whose ASO titres were < 200, and 19 of 134 sera with ASO titres ⩾ 200 whose anti-DNAase B titres were below the upper limit of normal. Among the sera in which a raised ASO was not confirmed by a raised anti-DNAase B titre, or vice versa, 20 of 44 had type specific antibody to types 5, 18, or 58 which suggested possible recent infection with these types in this outbreak.

Table 4 shows the relationship of ASO titre and anti-DNAase B titre with the presence of type-specific antibody to types 5, 18 or 58 and to type 5 considered alone. The presence of type-specific antibodies of any of the three types in 5 of 42 (12%) sera, without evidence of recent streptococcal infection (ASO < 100, anti-DNAase B < 250) probably indicates the 'background level' of type-specific antibodies to these three types in the community. Only 2 of 42 (5%) of these sera had type 5 antibodies. Bactericidal antibodies are known to persist for many years in man (Lancefield, 1959), but a small survey among workers in our laboratory revealed that only 1 out of 10 had antibody to types 5 or 18 and none had antibody to type 58. Table 4 shows that the percentage of sera with type-specific antibody was appreciably higher than this background level in groups with raised ASO titres, but low anti-DNAase B titres. Among sera with raised anti-DNAase B titres, but low ASO titres only those with ASO titres between 100 and 200 showed a high percentage of type-specific antibodies. Among the sera with ASO titres < 100 and anti-DNAase B titres > 250, only 1 of 8 had type-specific antibody to type 5, indicating that a raised anti-DNAase B titre may not be significant if the ASO is < 100.

Table 5 shows the effect of combining the results of ASO and anti-DNAase B

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Table 5. *Percentage of sera with raised ASO and/or anti-DNAase B titres*

Antibody titre	All sera (except samples from rheumatic fever patients)	Sera from patients with TS positive for type 5 M	Sera with type 5 antibody
ASO > 200	33·3	43·0	55·5
Anti-DNAase B > 250	37·9	37·5	66·9
ASO and/or anti-DNAase B raised	52·3	62·5	83·3
Anti-MAP > 20	47·0	75·0	75·0

TS = throat swab.

Table 6. *Relationship of ASO and anti-MAP titre with the presence of type-specific antibodies*

Anti-MAP titre	Sera with antibody to type 5 out of total sera with ASO titre of:			Sera with antibody to types 5, 18 or 58 out of total sera with ASO titre of:		
	< 100	100–200	> 200	< 100	100–200	> 200
> 80	0/0 (0)	0/0 (0)	11/14 (79)	0/0 (0)	0/0 (0)	13/14 (93)
20–80	1/12 (8)	6/21 (28)	9/22 (41)	2/12 (16)	8/21 (38)	12/22 (55)
< 20	2/40 (5)	6/21 (28)	1/12 (8)	6/40 (15)	8/21 (38)	2/12 (16)

Figures in parentheses indicate the percentages of sera with type-specific antibody in each group.

tests in three categories of sera. In all these categories the results of the combined test gave percentages of sera with evidence of recent streptococcal infection, 15–25% greater than either test alone. This was particularly striking among the sera with type-specific antibody to type 5. Only 55·5% of these had raised ASO titres whereas a combination of the ASO and anti-DNAase B results suggested that 83·3% had suffered a recent streptococcal infection.

Correlation of ASO with anti-MAP titres

The anti-MAP titres of all the sera were plotted as a scattergram in four ASO categories as shown in Fig. 2. There was good correlation between the two antibody titres, in that there were very few sera (3 of 28) with anti-MAP titres > 20 which had ASO titres of < 50. There were also few sera with ASO titres > 200 which had anti-MAP titres of less than 20, and of these only 2 of 12 had type-specific antibody to types 5, 18 or 58 and only 1 of 12 had antibody to type 5 (see Table 6).

Fig. 2 also shows that there were 34 patients, other than the six with rheumatic fever, who had anti-MAP titres of 60 or greater. Of these 34 patients 22 had type-specific antibody to types 5, 18 or 58.

Correlation of anti-MAP titres with the presence of type-specific antibody

The number of sera with antibody to type 58 (4) and to type 18 (18) were too small for any comparison to be made, but 36 of 142 sera from R.A.F. Halton

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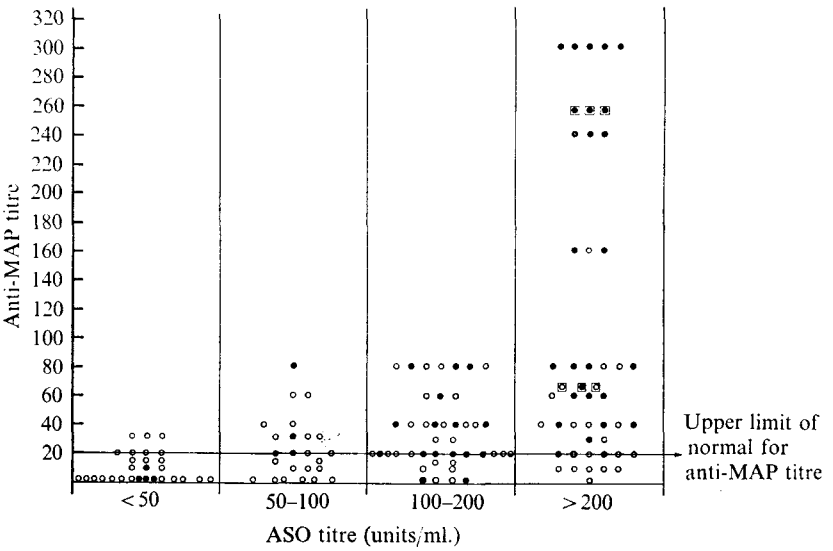

Fig. 2. The relationship of anti-MAP titre with ASO titre and the presence of type-specific antibody. ●, Denotes presence of antibody to M types 5, 18 or 58. ○, denotes absence of type-specific antibody to these types. □, rheumatic fever cases.

had type 5 antibody. Among these 36 sera only 55·5% had raised ASO titres (see Table 5) whereas 75% had elevated anti-MAP titres. The interrelationship between all three antibodies is shown in Table 6, where it is apparent that the highest proportion of sera with type 5 antibody occurs in the group with ASO titres > 200 and anti-MAP titres > 80. There were no anti-MAP titres > 80 among sera with ASO titres < 200.

Persistence of antibody to MAP

Follow-up bleedings were taken one year after the initial outbreak from ten of the cadets who had anti-MAP titres of 80 or greater. Nine out of the ten showed no decrease in anti-MAP titre. One showed a decrease from 80 to 40. The corresponding ASO titres in most cases showed a decrease, although one serum showed a rise from 430 to > 800 probably indicating a new infection.

DISCUSSION

The community at RAF Halton was not under constant surveillance for streptococcal infection before this outbreak. This investigation was begun only after a number of cases of rheumatic fever had been diagnosed. Throat swabs and sera from the rheumatic fever patients and other cadets were taken about 1 month after the start of the sore throat outbreak, so that there was no information about the types of streptococci prevalent at the very beginning. However, the persistence of type 5 in the succeeding 6 weeks, and the presence of type 5 antibody in many of the sera (43% of those with ASO ⩾ 200), including sera

from 4 out of 6 of the rheumatic fever patients, indicated that type 5 was probably responsible for the rheumatic fever, with some minor involvement of types 18 and 58 in the cases of sore throat.

Sera were obtained from a sample of cadets who were symptomless, had negative throat swabs and might have been thought not to have been involved in the outbreak. However, the antibody titres in these 'normal' sera were not significantly different from those in sera from boys who had suffered from sore throat (see Table 3). Only random samples of the group A streptococci isolated from cases of sore throat between 16 April and 13 May were typed, so that relatively few of the sera subsequently obtained could be matched with a throat swab result for the same patient. Also it was not practicable at the time to obtain paired sera from cadets other than those with rheumatic fever. These deficiencies leave considerable gaps in our information about the antibody responses of individual cadets but certain trends were apparent in the antibody responses for the community as a whole. The ASO response was in general rather weak, even among cadets from whose throats type 5 streptoccoci had been isolated, or who had type-specific antibody against this type in the sera. The anti-DNAase B response was in general better. However, it was only when the results of these two antibody tests were combined that a more realistic estimate of the percentage of cadets who had had a recent streptococcal infection was obtained, if the presence of antibody to type 5 could be taken as an indication of infection in this outbreak, where the 'background level' of type specific antibody to type 5 appeared to be between 5 and 10%.

The anti-MAP test is not an established antibody test in the diagnosis of streptococcal diseases. In our first report on the presence of this antibody in human sera (Widdowson *et al.* 1971) we stated that titres were higher in rheumatic fever (range 60–320) than in nephritis (range 0–80) or in sporadic uncomplicated streptococcal infection of the throat (range 0–80). We had not at that stage examined a large number of sera from cases of uncomplicated sore throat in a single outbreak. The result of tests done on sera from R.A.F. Halton showed that high anti-MAP titres were not confined to the sera of the cadets who developed rheumatic fever. Although the anti-MAP titres of the sera from the rheumatic fever patients were all high (> 60), 17 of 38 sera from cadets who had raised ASO titres but no sign of rheumatic fever, also had anti-MAP titres of greater than 60 (see Fig. 2). However, from other outbreaks, in which no cases of rheumatic fever occurred, we have examined sera from over 100 cases of uncomplicated sore throat caused by different streptococcal serotypes (e.g. types 6, 12 and 22) and found that, although the ASO and anti-DNAase B titres were comparable with or higher than those in the type 5 outbreak, the anti-MAP titres were in general much lower. For example only about 10% of sera with ASO titres greater than 200 from an outbreak of sore throat caused by type T12/M12 and T12/M22 strains had anti-MAP titres greater than 60. Moreover, about 70% of these sera had anti-MAP titres below the upper limit of normal (Widdowson, Maxted, Notley & Pinney, *in preparation*).

It therefore seems likely that the anti-MAP response is to a large extent

influenced by the infecting type. Although a high anti-MAP titre may not always be associated with rheumatic fever *per se*, perhaps the generally higher titres seen in rheumatic fever, compared with, for example, nephritis, are a reflexion of the influence of the type of streptococcus, both on the magnitude of the anti-MAP titre and the nature of secondary sequelae of streptococcal infection.

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