J. Hyg., Camb. (1985), **95**, 47–57
Printed in Great Britain

The production of pyrogenic exotoxins by group A streptococci

By GILLIAN HALLAS

Division of Hospital Infection, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT U.K.

(*Received* 8 *January* 1985; *accepted* 4 *March* 1985)

SUMMARY

Lancefield group A streptococci isolated from recent outbreaks and sporadic cases of scarlet fever were restricted to the following M types 1, 3, 4, 6, 12, 18, 22 and 66. These strains were examined for the presence of streptococcal pyrogenic exotoxins (SPE) types A, B and C by isoelectric focusing in polyacrylamide gels and by immunoprecipitation in agar gels. SPE B was produced by 70% of the strains and SPE C by 40%. SPE A could not be detected in these strains. In contrast, SPE type A was found in 4 of 10 strains, held by the NCTC, that had been isolated before 1940 from patients with scarlet fever. Nine of 12 recent isolates from patients with sore throat uncomplicated by a rash produced SPE C and 4 of these also produced SPE B.

INTRODUCTION

Scarlet fever is a complication of streptococcal sore throat. In the past there was a high mortality rate but generally it is now a mild infection. This could be due to a change in the organism, the host, or both. About 10000 cases are reported each year in England and Wales (Office of Population Censuses and Surveys, 1982) with both outbreaks and sporadic infections being seen in the community. This work was begun after a large outbreak of scarlet fever occurred in Shetland (Macgregor, 1980) coincidentally with an increase in the number of reported cases in the years 1978–80.

The characteristic skin rash of scarlet fever is believed to result from a combination of direct toxicity and a hypersensitivity reaction to one of three antigenically distinct pyrogenic exotoxins (SPE). These toxins, known as SPE A, B and C, are low-molecular-weight proteins with different isoelectric points (pI). SPE A has a mol. wt. of 8000 Daltons and a pI of 5·0, SPE B is 17000 Daltons with a pI of 8·4 and SPE C has a mol. wt. of 13000 Daltons and a pI of 6·7 (Watson, 1979). A strain of *Streptococcus pyogenes* (Lancefield group A) may produce none, one, two or all three toxins but it seems these toxins do not occur in strains of other Lancefield groups (Schlievert, Bettin & Watson, 1979). Surveys of recent clinical isolates have shown that SPE B is the most commonly produced toxin among strains isolated in the USA (Bloomster & Watson, 1982) and in Germany (Gerlach, Köhler & Knöll, 1982) but SPE C was produced by strains isolated from patients with scarlet fever in Japan (Kaneko *et al.* 1979).

This paper describes a study of the occurrence of these toxins among recent clinical isolates of group A streptococci, most of which were isolated from mild cases

of scarlet fever and a comparison made of the toxin profiles of these cultures with strains associated with scarlet fever before 1940 and deposited in the National Collection of Type Cultures (NCTC).

METHODS

Bacterial strains. Toxin-producing reference strains were a gift from Professor D. W. Watson, University of Minnesota. Strain 594 (serotype T8/25/IMP 19,M−) produces SPE A only. Strain T19 (serotype T15/17/19/23/47, M19) produces SPE B and a trace of SPE C. Strain T18P (serotype T−, M−) produces SPE C only. Strain NY5 (serotype T10, M−) produces all three toxin types (Watson, 1979). The following group A streptococci were selected from cultures isolated during 1980–3 and sent to the Division of Hospital Infection at the Central Public Health Laboratory for typing: organisms from patients with scarlet fever, also from sore throats uncomplicated by a rash and strains with the serotype T1,M1 isolated from fatal, septicaemic infections. Group C streptococci isolated from patients with a scarlatiniform rash were also examined. Isolates from cases of scarlet fever occurring before 1940 were obtained from the National Collection of Type Cultures, Central Public Health Laboratory, Colindale. There were ten Lancefield group A streptococci and one group L strain in this group.

Serotyping of strains. M and T typing was carried out by standard methods. *M type 1* was isolated during an outbreak of sore throat and scarlet fever at a school.

M type 3. Representatives of this type caused a large outbreak of scarlet fever in Shetland (Macgregor, 1980), a smaller outbreak at a school in England and were also the predominant isolate at a children's home in Scotland. Thirteen strains were cultured from patients in a general practice where there were cases of scarlet fever.

M type 4 streptococci were isolated from outbreaks at two schools, from three members of a family and from two siblings in a second family.

M type 6 were isolated from two girls in a single family.

Representatives of M types 22 and 66 were referred from Australia and New Zealand where they had caused scarlet fever. Single isolates of M types 1, 3, 6, 12, 18 and 22 were received from cases in the U.K.

Serotyping of NCTC isolates. Seven of the eight strains that could be T typed, failed to react with any of the routine M typing sera (Table 1). The possibility that some of these strains were M negative variants was suggested by the fact that strain 8330 (T type 22) was opacity factor negative (all M-positive strains with the T22 antigen are opacity factor positive). Cultures lacking an M antigen will not survive in fresh human blood and have a lower content of M-associated protein (MAP; Hallas & Widdowson, 1983) than M-positive cultures. Six strains failed to survive in human blood (Table 1) and also had low MAP titres ($\leqslant 60$). Four strains multiplied in human blood and the survivors from strain 8232 were typed as M type 47. The other two opacity factor positive strains could not be M typed.

Antisera. Specific antisera to the three toxins SPE A, B and C were kindly donated by Professor Watson. 'Scarlet fever' antitoxin was obtained from the Wellcome Foundation Ltd, Beckenham, Kent. The antitoxin, prepared in horses was formerly used in the treatment of scarlet fever.

Immunoglobulins were isolated from the Colindale-prepared antisera to the

Table 1. *Serotyping and toxin profiles of pre-1940 Lancefield group A cultures deposited with the National Collection of Type Cultures*

Catalogue number	Source	Serotype	Survival in human blood	MAP titres	Toxin profile
2218	SF 3 scarlatina, Aberdeen, 1926	T1, M−, OF−	−	40–80	B
3359	Strain Dochez used at Wellcome Laboratories for antitoxin production; deposited in 1931	T−, M−, OF−	−	NT	AC
8198	SF 130 scarlet fever in Manchester	T1, M1, OF−	+	160–320	A
8232	Scarlet fever in 1932	T19/15/17/23/47, M47, OF−	+	160–320	AB
8303	Scarlet fever in Liverpool (Griffith, 1934)	T−, M−, OF−	−	10–20	C
8318	Strain Blackmore scarlet fever outbreak before 1934	T11, M−, OF−	−	10–20	Negative
8324	SF 4 outbreak of scarlet fever about 1932	T8/25/IMP 19, M−, OF+	+	10–20	B
8328	SF 40 scarlet fever in 1932	T5/27/44, M−, OF+	+	40–80	C
8330	Scarlet fever (Griffith, 1934)	T22, M−, OF−	−	20–40	BC
8332	Scarlet fever	T10, M−, OF−	−	10–20	ABC
6396	Scarlet fever case in Victoria, Australia, 1938 Lancefield group L				Negative

NT, not tested.

SPE A and B toxins by ammonium sulphate precipitation as described by Harboe & Ingild (1973).

*Isolation of toxins from reference strains.*SPE A. Strain 594 was grown in one litre of chemically defined medium (Van de Rijn & Kessler, 1980) for 16 h at 37 °C. Proteins were precipitated from the Millipore-filtered culture supernate by the addition of solid ammonium sulphate to give 70% saturation. After storage overnight at 4 °C, the precipitate was collected by centrifugation. It was then redissolved in 5 ml of distilled water and dialysed against glycine (1%, w/v). SPE A was further purified by sucrose-density gradient isoelectric focusing. Fractions of 2 ml were run off the bottom of the column. The presence of SPE A was detected in the column fractions by double diffusion in a phosphate-buffered-saline agarose gel with scarlet fever antitoxin. Positive fractions were pooled, dialysed against distilled water and stored at −20 °C.

SPE B was prepared from strain T19 grown in dialysate medium of Todd–Hewitt Broth (Difco Ltd, Chertsey, Surrey) with Neopeptone (Difco; 2% w/v) for 16 h at 37 °C. The dialysate medium was prepared by pumping the broth through a Model DC 2 hollow fibre system (Amicon Ltd, High Wycombe, Bucks) using a cartridge with a mol. wt. cut off of 30000. The filtrate was collected, dispensed into aliquots as required and sterilized at 121 °C for 15 minutes. This strain did not produce detectable toxin when grown in the chemically defined medium used for strain 594. Proteins were precipitated from the culture supernate with ammonium sulphate (70% saturation) or by the addition of absolute ethanol. Ethanol precipitation was carried out as described by Kim & Watson (1970). The resulting precipitate was dissolved in acetate-buffered saline (0·005 M acetate buffer pH 4·0 containing 0·15 M-NaCl) and dialysed against 1% glycine.

Ammonium sulphate-precipitated material was dissolved in distilled water and dialysed against distilled water overnight. This solution was treated by batch absorption for 60 min at 4 °C with DE 52 diethylaminoethyl cellulose, which had been previously equilibrated to pH 7 with Tris buffer. The cellulose was removed by centrifugation and the supernate was dialysed against 1% glycine. Sucrose-density gradient isoelectric focusing was performed on the dialysed material using a pH gradient of 6·0–9·5. The focusing fractions were monitored for the presence of SPE by gel diffusion using the reference anti SPE B serum.

Preparation of antisera to SPE. Toxin-containing isoelectric focusing fractions were sterilized by filtration through a Millipore filter. A 0·9 ml aliquot was mixed with 0·1 ml aluminium hydroxide adjuvant (Alu Gel S, Serva Ltd) and left at 4 °C overnight before injection. Rabbits were injected intramuscularly at 1 and 14 days. After 2 weeks they were then injected subcutaneously once a week for 3 weeks and sampled after a further week. Vaccination was continued at weekly intervals if there was no antibody response after the first course.

In addition to the above procedure, attempts were made to produce an antiserum to SPE C by injection of immunoprecipitates. Crude ethanol-precipitated toxin was prepared from strain T18P using the method described for SPE B. At a concentration of 400 mg/ml a precipitin line was formed in gel diffusion when tested against the reference anti C serum. The concentration of crude SPE C was 10 times the amount required to demonstrate the presence of SPE A and SPE B with the homologous reference antiserum. The gel slide was washed with saline for

2 days to remove the soluble proteins. The portion of the agar containing the precipitin line was cut out and homogenized with 1·0 ml of saline. This material was combined with 0·1 ml of adjuvant and then injected intramuscularly into rabbits.

Isolation of crude toxin preparations from test strains. Strains were grown in 50 ml of dialysate medium. The crude toxin was precipitated from the culture supernate with ethanol and redissolved in acetate-buffered saline as described for SPE B. After dialysis against distilled water, the preparations were lyophilized. Approximately 180 mg was obtained per 50 ml of original culture.

Detection of toxins in crude preparations was by double diffusion in a gel of agarose (1% w/v) in phosphate-buffered saline (Dulbecco 'A', Oxoid Ltd). Samples were tested for the presence of SPE A and SPE B at a concentration of 40 mg/ml. At this concentration, solutions of stock strains NY5, 594 and T19 reacted with the appropriate antisera. Any samples that gave a line of precipitation with the Colindale-prepared antisera were also tested against the reference antisera obtained from Professor Watson. Solutions of 400 mg/ml were required to test for SPE C by gel diffusion. At this concentration, the stock strains NY5 and T18P gave strong precipitation lines with the reference anti SPE C serum.

Samples were also monitored for SPE production by polyacrylamide gel electrofocusing using LKB Ampholine PAG plates pH range 3·5 to 9·5. Samples of 7·5 mg were dissolved in 0·03 ml distilled water and 0·015 ml aliquots were applied to the gel on filter paper strips. Staining of the gels was done according to the manufacturer's instructions.

RESULTS

Isoelectric focusing studies

Five protein-staining bands were obtained when ethanol-precipitated toxin from the reference strain NY5 was examined by isoelectric focusing in polyacrylamide gels. This strain is reported to produce all three toxins. The bands focused at positions in the gel corresponding to pH values of 8·5, 7·8, 5·5, 5·2 and 5·0. SPE A toxin purified from strain 594 by column isoelectric focusing, contained two protein bands with pI values of 5·2 and 5·0. SPE B, purified from strain T19 by alcohol precipitation and column isoelectric focusing, formed a single band corresponding to a pH of 8·5.

Comparison of the detection of SPE by isoelectric focusing and gel diffusion

Rabbit antisera were successfully prepared against SPE A and SPE B. There was no detectable antibody response to SPE C when alcohol-precipitated toxin was used as a vaccine nor when immunoprecipitates were injected.

A summary of the detection of SPE A and SPE B by isoelectric focusing and gel diffusion with three antisera to SPE B and two antisera to SPE A are shown in Table 2. Strains were screened for SPE A and SPE B with the Colindale-prepared antisera and those giving positive reactions were then tested with the reference antisera. Toxins presumptively identified by gel diffusion tests were confirmed by polyacrylamide-gel electrofocusing. Sixty two group A strains were tested. Anti SPE B serum, prepared by injecting ammonium sulphate-precipitated toxin,

Table 2. *Detection of SPE A and SPE B by gel diffusion and isoelectric focusing*

Number of strains	Anti A* 2372	Reference anti A	Anti B* 2525	Anti B† 2564	Reference anti B	IEF
14	−	NT	−	−	NT	NT
2	−	NT	−	−	+§	NT
4	−	NT	+	+	+	NT
3	−	−	−	−	−	Negative
2	−	NT	−	−	NT	Negative
1	−	NT	−	−	+§	Negative
2‡	−	NT	+	−	−	Negative
1	+	−	+	−	NT	Negative
1	−	NT	−	−	NT	B } Weak
1	−	NT	−	+	NT	B } Weak
1	−	NT	+	−	−	B } Weak
1	−	NT	+	−	+	B
22	−	NT	+	+	+	B
1	−	−	+	+	+	B
2	+	−	+	+	+	B
1	+	+	+	−	−	A
1	+	±	−	−	−	A
1	+	+	+	−	−	A, B
1	+	+	+	+	+	A, B

NT, not tested.
* Antiserum prepared from ammonium sulphate-precipitated toxin.
† Antiserum prepared from ethanol-precipitated toxin.
‡ Both strains isolated from the same outbreak.
§ Tested at 10× concentration.

reacted with 37 strains. Fewer positive results were obtained with the serum prepared with ethanol-precipitated vaccine (31) or with the reference anti SPE B serum (34 strains). Colindale-prepared anti SPE A serum detected 7 positives. The presence of SPE A was confirmed with the reference serum and isoelectric focusing in 4 strains.

SPE production in isolates from recent cases of scarlet fever

A total of 40 strains of group A streptococci isolated from cases of scarlet fever were tested for the production of SPE A, B and C (Table 3). Seven strains were examined from sporadic cases of infection in the U.K. Where outbreaks of infection occurred more than one strain was examined and 28 isolates were tested from 10 incidents. The toxin profiles of strains from the same outbreak were identical in 8 different episodes. In 2 outbreaks, in which 2 strains were examined from each group, SPE B was isolated from 1 strain but SPE B and C were detected in the second isolate. SPE B was the most frequently detected toxin in all the isolates, occurring singly in 19 strains and in combination with SPE C in 9. SPE C was found alone in 7 isolates. None of these strains appeared to produce SPE A when examined by isoelectric focusing or gel diffusion. No toxin was detected in the 5 strains examined from 2 outbreaks.

The 40 isolates listed in Table 3 represented 8 different M types. When the serotype of the infecting strain is compared with SPE pattern all the M type 1

https://doi.org/10.1017/S0022172400062276 Published online by Cambridge University Press

Table 3. *Distribution of SPE types in 40 cultures of Lancefield group A streptococci from cases of scarlet fever*

'Outbreak'	M type	No. of strains examined	Toxin profile
Shetland	3	5	B
Children's home	3	3	B
	12	1	B
	22	1	BC
School 1	3	2	Negative
School 2	4	2	C
School 3	4	2	B (BC)
School 4	1	3	B
General practice	3	3	Negative
Family outbreaks	6	2	C
	4	2	B (BC)
	4	2	BC
Single isolates	1	1	B
	3	1	B
	3	1	BC
	6	1	C
	18	1	C
	22	1	C
	22	1	BC
Isolates referred from			
Australia	22	1	BC
New Zealand	22	2	B
	66	2	B (BC)

strains produced SPE B and the M type 6 strains produced SPE C. However the numbers are small.

Three strains of group C streptococci (*S. equisimilis*), isolated from three patients with scarlatiniform rashes, were sent to us from a north London hospital. SPE toxins were not detected in the 2 strains examined, which confirms previous observations (Schlievert, Bettin & Watson, 1979) that SPE toxins are only produced by group A streptococci.

SPE production in isolates from cases of scarlet fever before 1940

The results are shown in Table 1. SPE C was detected in 5 strains. The reaction obtained with strains 3359 and 8303 was weak but reproducible in 2 experiments which suggested that SPE C is produced in trace amounts only in these strains. Tested at the same concentration, the other 3 strains gave strong precipitin lines.

SPE A was formed as a single toxin in strain 8198 and in combination with SPE B or SPE C in 2 strains. Strain 8332 appeared to produce all 3 toxin types. SPE B was detected as a single toxin in 2 strains. The group L strain produced none of the SPE types.

Table 4. *Distribution of SPE types in isolates of Lancefield group A streptococci from uncomplicated throat infections*

M type	No. of strains examined	No. of strains with toxin profile				
		A	B	C	BC	Negative
1	1	—	—	—	—	1
2	1	—	—	1	—	—
4	2	—	—	—	2	—
6	3	—	—	2	—	1
9	1	—	1	—	—	—
12	2	—	—	1	1	—
22	2	—	—	1	1	—
Total	12	—	1	5	4	2
Percentage	100	—	8	42	33	17

SPE production in strains isolated from uncomplicated throat infections

Twelve strains were examined in this group including 4 strains with the serotypes M 6, 12 or 22 isolated from pupils during an outbreak of streptococcal sore throat at a preparatory school (Table 4). All 4 strains produced a single pyrogenic toxin, SPE C. Production of SPE B as a single toxin was less common in these isolates compared with the strains from cases of scarlet fever. The combination SPE B with SPE C occurred in 33% of the isolates.

SPE production in strains isolated from fatal infections

Our results had shown that some of the group A isolates from the more severe (pre-1940) cases of scarlet fever were SPE A producers whereas none of the strains associated with the current milder form of the disease produced this toxin type. This suggested that SPE A may be associated in some way with virulence of the organism. Fatal, septicaemic streptococcal infections in previously healthy people are most often caused by M type 1 serotypes. Three such strains were examined for SPE A and B. None of the strains produced SPE A but 2 strains formed SPE B.

DISCUSSION

In this study, M type 3 and M type 4 streptococci appeared to be the most common types associated with recent cases of scarlet fever in the U.K. and were the predominant isolates in 4 outbreaks. M type 1 and M type 6 streptococci were isolated from single outbreaks.

Perks & Mayon-White (1983), in a study of scarlet fever in the Oxford region found that 48% of the cultures were M type 4 and M type 3 was cultured from 20% of their patients. Some 30 years ago representatives of type 4 were isolated more frequently from patients with scarlet fever than from those with uncomplicated tonsillitis (Report 1954, 1957). In an international survey of the distribution of the serotypes of group A streptococci (Parker, 1967), representatives of M type 3 and M type 4 were found to be associated with scarlet fever in several countries. Representatives of M type 1 were isolated from patients with scarlet fever in the

U.S.S.R. and the Netherlands; type 22 was associated with scarlet fever only in the D.D.R. In this study, M type 22 streptococci were found among strains referred from Australia and New Zealand and also in two cases in the U.K.

Some difficulty was experienced in raising antisera to the SPE toxins in rabbits. These toxins are low molecular weight proteins and do not appear to be highly antigenic (Watson & Kim, 1970). SPE B was the least difficult and antisera were prepared to both ammonium sulphate-precipitated toxin and alcohol-precipitated toxin. No detectable antibodies were formed when alcohol-precipitated SPE A or SPE C were used as vaccines.

Precipitin lines were obtained with antisera prepared against ammonium sulphate-precipitated SPE A and SPE B that could not be confirmed with the reference antisera. Presumably, ammonium sulphate precipitates all the extracellular proteins from the culture supernate. Using the alcohol procedure, many extracellular products are removed by the differential solubility between ethanol and acetate-buffered saline (Watson, personal communication). This is important in the preparation of SPE B because proteinase produced by many of the SPE B producers has a pI close to B toxin.

Five protein-staining bands were obtained when alcohol-precipitated NY5 toxins were examined by polyacrylamide-gel isoelectric focusing. The band focusing closest to the cathode was identified as SPE B for the following reasons: the band focused at about pH 8·5 in most experiments which is in good agreement with the published values for the pI of SPE B. Secondly, this band was absent in the reference SPE A producer strain (594). Thirdly, SPE B vaccine prepared by alcohol precipitation and further purified by column isoelectric focusing, focused in the same position in the gel as the cathodic NY5 protein band. SPE A purified from strain 594 by alcohol precipitation and column isoelectric focusing was heterogeneous when separated on polyacrylamide isoelectric focusing gels. Two protein bands were formed (pI 5·0 and 5·2) which focused in the same position as the two bands nearest the anode in the NY5 strain. The presence of both bands was taken as being indicative of SPE A in the examination of toxin profiles from other strains. It was considered unlikely that the protein band focusing at about pH 7.8 was SPE C because of the discrepancy between this value and its previously reported pI of 6·7. Also this band was present in strain 594 which does not produce SPE C.

Some strains that were assumed to be SPE C producers by gel diffusion studies were examined by isoelectric focusing. An additional band which focused to the anode side of the pH 7·8 band was seen in some strains.

In one experiment, a similar band was faintly visible in NY5. However, these results could not be considered as conclusive, so gel diffusion studies with the reference anti C serum were relied upon to detect the presence of SPE C.

Studies of the toxin profiles showed that there was a high prevalence of SPE B producers (70%) among recent isolates from scarlet fever patients. Bloomster & Watson (1982) found that 12 of 14 clinical isolates of group A streptococci from the USA were SPE B producers.

Three strains formed a precipitin line in gel with the Colindale-prepared anti SPE A serum, but the presence of SPE A was not confirmed with the reference serum or by isoelectric focusing. These strains were therefore considered to be

SPE A negative. A sample of anti SPE A serum was sent to Professor Watson who showed that the Colindale-prepared serum gave a good line of identity comparable with the Minnesota serum and that it did not contain antibodies to SPE B or SPE C. However the Colindale serum probably contains some cross-reacting antibodies in addition to anti SPE A as some false positives were obtained. SPE A was detected in four isolates derived from scarlet fever cases before 1940. Tested under the same conditions, SPE A did not appear to be produced by recent isolates. Either they do not produce SPE A or they produce small amounts of toxin in comparison with the reference strains (against which the tests were standardized) and this was not detected in the concentrations used in these experiments. It is interesting to note that SPE A was most probably the first reported toxin (Hooker & Follensby, 1934). There is evidence to suggest that there has been a decline in the incidence and severity of streptococcal infections (Quinn, 1982); this study has shown that there appears to be a difference in the type of SPE produced by recent isolates from scarlet fever cases and some of the strains isolated before 1940.

I wish to thank Professor D. W. Watson for providing the reference strains and antisera and also Dr G. Colman for his helpful advice in the preparation of the manuscript.

REFERENCES

BLOOMSTER, T. G. & WATSON, D. W. (1982). Recent trends in streptococcal pyrogenic exotoxin production by group-A streptococci. In *Basic Concepts of Streptococci and Streptococcal Diseases* (ed. S. E. Holm & P. Christensen), pp. 139–141. Chertsey: Reedbooks Ltd.

GERLACH, D., KÖHLER, W. & KNÖLL, H. (1982). Comparison of erythrogenic toxins produced by *Streptococcus pyogenes*. In *Basic Concepts of Streptococci and Streptococcal Diseases* (ed. S. E.Holm & P. Christensen) pp. 137–138. Chertsey: Reedbooks Ltd.

GRIFFITH, F. (1934). The serological classification of *Streptococcus pyogenes*. *Journal of Hygiene* **34**, 542–584.

HALLAS, G. & WIDDOWSON, J. P. (1983). The relationship between opacity factor and M protein in *Streptococcus pyogenes*. *Journal of Medical Microbiology* **16**, 13–26.

HARBOE, N. & INGILD, A. (1973). Immunization, isolation of immunoglobulins, estimation of antibody titre. *Scandinavian Journal of Immunology* **2**, Suppl. no. 1, p. 162.

HOOKER, S. B. & FOLLENSBY, E. M. (1934). Studies on scarlet fever. II. Different toxins produced by hemolytic streptococci of scarlatinal origin. *Journal of Immunology* **27**, 177–193.

KANEKO, Y., MURAI, T., YATA, J., OKUDA, R., TUPASI, T. E., REYES, A. L., RAMOS, C. P. & ANTONIO-VELMONTE, M. (1979). Streptococci prevalent in Manila, the Philippines. In *Pathogenic Streptococci* (ed. M. T. Parker), pp. 110–111. Chertsey: Reedbooks Ltd.

KIM, Y. B. & WATSON, D. W. (1970). A purified group A streptococcal pyrogenic exotoxin. Physicochemical and biological properties including the enhancement of susceptibility to endotoxin lethal shock. *Journal of Experimental Medicine* **131**, 611–628.

MACGREGOR, J. D. (1980). Scarlet fever in Shetland: December 1979 to April 1980. *Communicable Diseases Scotland* **21**, vii–ix.

OFFICE OF POPULATION CENSUSES AND SURVEYS. Communicable Disease Surveillance Centre of the Public Health Laboratory Service (1982). In *Communicable Disease Statistics* Series MB2 No. 9, pp. 6–7. London: HMSO.

PARKER, M. T. (1967). International survey of the distribution of serotypes of *Streptococcus pyogenes* (group A streptococci). *Bulletin of the World Health Organisation* **37**, 513–527.

PERKS, E. M. & MAYON-WHITE, R. T. (1983). The incidence of scarlet fever. *Journal of Hygiene* **91**, 203–209.

QUINN, R. W. (1982). Epidemiology of group A streptococcal infections – their changing frequency and severity. *Yale Journal of Biology and Medicine* **55**, 265–270.

REPORT, (1954). *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **13**, 171–174.

REPORT (1957). *Monthly Bulletin of the Ministry of Health and Public Health Laboratory Service* **16**, 163–172.

SCHLIEVERT, P. M., BETTIN, K. M. & WATSON, D. W. (1979). Production of pyrogenic exotoxin by groups of streptococci: association with group A. *Journal of Infectious Diseases* **140**, 676–681.

VAN DE RIJN, I. & KESSLER, R. E. (1980). Growth characteristics of group A streptococci in a new chemically defined medium. *Infection and Immunity* **27**, 444–448.

WATSON, D. W. (1979). Characterisation and pathobiological properties of group-A streptococcal pyrogenic exotoxins. In *Pathogenic Streptococci*, (ed. M. T. Parker), pp. 62–63. Chertsey: Reedbooks Ltd.

WATSON, D. W. & KIM, Y. B. (1970). Erythrogenic toxins. In *Microbial Toxins*, vol. III. *Bacterial Protein Toxins* (ed. T. C. Montie, S. Kadis and S. J. Ajl), pp. 173–187. New York: Academic Press.