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# Serological characterization of group-A streptococci associated with skin sepsis in meat handlers

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## SUMMARY

A series of outbreaks of skin sepsis among meat handlers in England during 1974 and 1975 afforded an opportunity to study the group-A streptococci commonly isolated from the lesions. Few of these streptococci could be M typed with existing antisera. Intensive study of strains from two outbreaks of sepsis in one abattoir in Shropshire led to the recognition of three new provisional M types. The streptococci were first sorted according to T-typing pattern and ability to produce opacity factor. Opacity-factor producing strains with the same T pattern were then screened for inhibition of opacity production by the sera of convalescents from the same outbreak. Finally, M antisera were made in rabbits against representative cultures.

Sera against the three new provisional types were used to re-examine streptococci from 20 other outbreaks or incidents of sporadic infection among meat handlers. This increased the proportion of typable strains from 3 % to 55 %. Two of the new provisional types (nos. 2015 and 1658; both T25/Imp 19, opacity-factor positive) were confined to the Shropshire outbreak, but the third (no. 2681; T14, opacity-factor negative) was found among strains from meat workers in eight other geographically distinct areas. In all, 31 % of 131 distinct strains from meat workers, but less than 1 % of 2816 strains from other British sources, belonged to provisional type 2681. Thus, in Britain, one M-type of group-A streptococcus appears at present to be almost exclusively associated with sepsis in meat workers.

## INTRODUCTION

A number of outbreaks of skin sepsis, caused by group-A streptococci among workers with raw meat, were reported to the Public Health Laboratory Service (PHLS) between June 1974 and November 1975. Brief accounts of most of these have been published (Public Health Laboratory Service, 1975*a* and *b*) and one of

us (C.A.M) carried out a detailed study of two outbreaks in an abattoir in Shropshire.

Study of the epidemiology of these outbreaks was greatly hampered by the difficulty of typing the streptococci isolated from the skin lesions. Very few of the strains appeared to belong to internationally accepted M-types for which we had precipitating antisera. In this respect, the streptococci appeared to resemble the group-A strains associated with impetigo in children in Britain (Parker, Tomlinson & Williams, 1955), and in many other countries (Wannamaker, 1970), and occasionally in groups of adults subject to frequent skin trauma, such as members of sports and athletic teams (Glezen, de Walt, Lindsay & Dillon, 1972).

Inability to M-type 'skin' streptococci is partly attributable to the poor antibody response to many of them in rabbits, and the consequent difficulty in preparing a comprehensive set of typing antisera for them. The fact that streptococcal skin sepsis is usually sporadic also makes it difficult to choose strains for antiserum production that are likely to represent widely distributed serotypes. The sets of strains from a series of apparently distinct epidemics among meat-handlers thus provided a valuable starting-point for a study of 'skin' types. We therefore searched for new types among strains from the two Shropshire outbreaks, making use of the facts that (1) members of the same M type almost invariably have the same T-typing pattern (Williams & Maxted, 1953), (2) production of opacity factor (OF) occurs in all members of some M types but is absent from all members of the remaining types (Maxted *et al.* 1973*b*), (3) the serological specificity of the OF exactly parallels that of the M antigen (Widdowson, Maxted & Grant, 1970), and (4) human convalescent sera often contain neutralizing antibody for the OF of the infecting streptococcus (Maxted, Widdowson & Fraser, 1973*a*). We then extended our study to a collection of strains from 20 other incidents of infection in meat handlers.

## MATERIALS AND METHODS

### *Epidemiological information*

The abattoir in Shropshire was visited on several occasions by one of us (C.A.M), a general practitioner (Dr Duncan MacGregor) or an Environmental Health Officer (Mr Charles Jones), and specimens were collected from patients and environmental sources. Information about the other outbreaks of sepsis was provided by PHLS and hospital microbiologists for the weekly Communicable Diseases Report of the PHLS, or accompanied streptococci sent to the Streptococcus Reference Laboratory for typing.

### *Streptococcal strains*

In all, 144 strains of group-A streptococci from workers or environmental sites in abattoirs and other meat factories, or from retail butchers, were received. Strain R68/3354, the type strain for provisional M type (PT) 3354, and strain SF/130/13 (no. NCTC8198) the type strain for M-type 1, were used in bactericidal tests.

*Sera*

M- and T-type antisera were prepared in rabbits as described by Williams (1958). Anti-OF sera were either (1) stock M antisera that had been found to have opacity neutralizing activity against streptococci of corresponding M type, (2) rabbit antisera prepared specifically for the opacity-neutralization test (Maxted *et al.* 1973*b*), (3) pooled antisera prepared by intraperitoneal inoculation of streptococci into guinea-pigs (Maxted, unpublished), or human sera found to have type-specific opacity-neutralizing activity (Maxted *et al.* 1973*a*).

Precipitating antisera were available for OF-negative M types 1, 3, 5, 6, 12, 14, 15, 17, 18, 19, 23, 24, 26, 29, 30, 31, 33, 36, 37, 39, 41, 43, 46, 47, 50, 51, 52, 53, 54, 55, 56 and 57; and precipitating and anti-OF sera for OF-positive M types 2, 4, 9, 11, 22, 25, 27, 28, 48, 49, 58, 59, 60, 61, 62, 63 and 66. Thus, we were in a position to detect all the internationally recognized M types except nos. 8, 13, 44, 64 and 65. In addition, we had antisera against seven OF-positive provisional types that had been first recognized in this laboratory and designated as follows: PT 3354, PT Truro, PT 2773, PT Constable, and PT 346 (precipitating and anti-OF sera); and PT 3875 and PT Nottingham (anti-OF sera only).

Sera from patients had been collected about 4 weeks after the onset of clinical disease and were stored at −20 °C before being examined.

The horse serum used in opacity tests was Burroughs Wellcome Horse Serum (no. 5 HSO2). Batches that had given good opacity reactions with culture supernatants of OF-positive streptococci were chosen.

*Growth medium for streptococci*

'Broth' was Todd-Hewitt Broth (Difco) to which 2% (w/v) of Neopeptone (Difco) had been added.

*Typing of streptococci*

T-typing was by slide agglutination (Williams, 1958); broth cultures incubated overnight at 30 °C were used. M-typing by precipitation was performed in agar gel by a double-diffusion method (see Rotta *et al.* 1971); hot-acid extracts (in 0·2 N-HCl at 100 °C for 10 min) were made from deposits from broth cultures that had been incubated overnight at 37 °C.

*The serum-opacity reaction and its neutralization*

All streptococcal strains were first tested for the production of OF by the method of Maxted *et al.* (1973*b*).

The OF-neutralization test was performed in one of two ways: (1) by incorporating a hot-acid extract or broth supernatant of an OF-positive streptococcus, together with horse serum, in agar gel, pouring this as a thin layer on a large glass slide, and placing loopfuls of antiserum on the surface (Maxted *et al.* 1973*b*); a circular area of clearing of opacity was a positive reaction; or (2) by incorporating an anti-OF antiserum and horse serum in the agar, and placing loopfuls of culture extract or supernatant on the surface (Maxted *et al.* 1973*a*); in this case, an area of opacity was a positive reaction.

Method 1 was used for the OF typing of streptococcal strains by means of our set of anti-OF antisera. It was also used to screen human sera for anti-OF antibody against an untypable streptococcal strain. When a serum containing such an antibody had been identified, it was used by method 2 to examine sets of untypable streptococci for other members of the same type.

*The indirect bactericidal test*

This test (Maxted *et al.* 1973*a*) was used to detect 'bactericidal' M antibody in the sera of human subjects.

## RESULTS

### *Clinical and epidemiological observations*

*The Shropshire outbreaks*

Between 22 June and 29 July 1974, 30 of 43 workers in an abattoir suffered from local sepsis of the hands and arms, usually after superficial injuries at work. When the abattoir was first visited on 30 July 1974, group-A streptococci were isolated from six of eight swabs from lesions, and from the throat of three workers who gave a history of recent sore throat. All the employees were given a 10-day course of oral penicillin or erythromycin and the abattoir was closed for 8 days while it was being disinfected. About 6 weeks after it was reopened, one of the men was being examined for a possible fractured finger and was found to have a septic wound; this had been present for 4 weeks but had been concealed for fear of financial loss. A swab of the wound yielded group-A streptococci, and enquiry revealed that, in all, 17 workers had had septic lesions since the abattoir had been reopened. Group-A streptococci were isolated from 12 more lesions and from the throat of five and the nose and throat of one worker. They were isolated also from several environmental sites in the abattoir, including chain-mail gloves, scabbard holders, and a sink. All the employees were given a 10-day course of penicillin or erythromycin, and the 17 with infected lesions or positive nose or throat cultures were excluded from the abattoir. The infected workers were re-swabbed 48–72 h after completion of the antibiotic course, and group-A streptococci were isolated from the throat of three and from a new lesion in one, and these four patients were again treated with penicillin. Attempts were made to improve the hygiene of the employees by ensuring that the paper towels and hand cleansers provided in the washrooms were used, by arranging for a health visitor to make periodic visits to the abattoir, and by appointing a general practitioner as their medical officer. These measures appeared to be successful, but about 14 months later a further smaller self-limiting outbreak occurred affecting 11 of 40 employees.

*Other reported outbreaks*

In the other reported outbreaks (Table 1) the skin lesions were described variously as acute sepsis in previously observed cuts and abrasions, as 'impetigo', and as 'erysipeloid'. In at least three outbreaks the predominant type of lesion was said to be impetigo-like. A number of the lesions had been present for several

Table 1. *Eight reported outbreaks of skin sepsis in meat handlers*

| Incident no. | Geographical location | Date of onset | Type of establishment | Species of animal processed | Attack rate* | No. of distinct cultures† submitted for typing |
|---|---|---|---|---|---|---|
| 1 | Shropshire | June 1974 | Same abattoir | Cattle, sheep | 30/43 | 31 |
| 2 | Shropshire | Nov. 1975 | | | 11/40 | 11 |
| 3 | Kent | Oct. 1975 | Two abattoirs | Sheep | 30/37 | 18‡ |
| 4 | Northamptonshire | Sept. 1974 | Abattoir | Pigs, cattle, sheep | 28/41 | 16 |
| 5 | West Midlands | Aug. 1975 | Abattoir | Pigs, cattle, sheep | 9/17 | 10 |
| 6 | Greater London | Oct. 1974 | Food-processing factory | Cattle, sheep | 7/82 | 2 |
| 7 | Nottinghamshire | Oct. 1974 | Meat-packing factory | Cattle, sheep | 9/12 | 1 |
| 8 | Warwickshire | Sept. 1974 | Abattoir | Sheep | 15/30 | 0 |

* Number of workers with skin sepsis/number at risk.
† Strains from different persons or distinct environmental sites.
‡ Includes one meat inspector and one retail butcher.

Table 2. *Other incidents of skin sepsis in meat handlers*

| Incident no. | Date of onset | Nature of incident | No. of distinct cultures* submitted for typing |
|---|---|---|---|
| 9 | Sept. 1974 | Infections among abattoir workers, retail butchers, and others | 11† |
| 10 | Nov. 1974 | Outbreak; meat-processing factory | 8 |
| 11 | Nov. 1974 | Four butchers and one cashier in two retail shops | 5 |
| 12 | Nov. 1974 | Outbreak; meat-processing factory | 4 |
| 13 | Dec. 1974 | Sporadic; abattoir | 3 |
| 14 | Dec. 1975 | Sporadic; abattoir | 2 |
| 15 | Nov. 1974 | Sporadic; food-processing factory | 2 |
| 16 | Nov. 1974 | Sporadic; abattoir | 1 |
| 17 | Nov. 1974 | Sporadic; abattoir | 1 |
| 18 | Sept. 1975 | Sporadic; abattoir | 1 |
| 19 | May 1975 | Outbreak; abattoir | 1 |
| 20 | Oct. 1975 | Sporadic; retail butcher | 1 |
| 21 | Nov. 1975 | Sporadic; retail butcher | 1 |
| 22 | Sept. 1974 | Sporadic; abattoir | 1 |

* Strains from different persons.
† Includes one environmental health officer.

weeks and some of these had proceeded to chronic ulceration. Local cellulitis, lymphangitis or lymphadenitis was present in some cases. Most of the lesions were on the hands and forearms, but a few lesions on the face or on normally covered parts of the body were described. One man had a primary lesion on the face on the same side as the shoulder on which he habitually carried meat. Most of the sufferers were slaughtermen or meat packers, but a few supervisory staff and lorry drivers were affected. Associated cases of sore throat were not reported in any of the outbreaks except the first in Shropshire.

Seven of the eight outbreaks began in the months June–October. The tendency for skin sepsis to occur in the late summer or autumn was known to many of the workers, and in several of the establishments this had been associated with the arrival of animals or carcasses from a particular source. When all the outbreaks were considered together, however, handling animals from a particular geographical area did not appear to be a common factor. It was observed that all the affected establishments processed sheep at some time, but that some did not process pigs and some did not process cattle.

One of the outbreaks affected two separate abattoirs in the same town at about the same time; the only apparent common factor was that the same meat inspectors worked in both, and that one of these was the first person to be affected. Field investigations in some of the outbreaks revealed obvious defects in hygiene, notably poor and inadequately maintained hand-washing facilities, but few clear indications were obtained of the source or mode of transmission of infection.

In all of the outbreaks in which a representative sample of lesions was examined bacteriologically, the predominent finding was of group-A streptococci, which were isolated from between one-half and four-fifths of the swabs examined. A smaller

and somewhat variable proportion of the swabs yielded *Staphylococcus aureus*. In the Warwickshire outbreak, only one swab – from a penicillin-treated patient – had been examined.

*Other incidents of infection*

Cultures of group-A streptococci were received from 14 incidents of infection other than the reported outbreaks (Table 2), all of them in different areas of the country. Many of these were sent in the months of November and December 1974, soon after accounts of the earlier reported outbreaks had appeared. Certain of the incidents were obviously outbreaks in abattoirs and meat-processing factories (e.g. nos. 10 and 12). Others appeared to be sporadic infections in similar establishments, but epidemiological information was often inadequate. Some infections in retail butchers were included; in one town (incident 11) there were five infections in two apparently unconnected butchers' shops, in which four butchers had lesions on the hands and a cashier had a sore on the thigh.

## *Bacteriological observations*

*Routine typing of streptococcal strains*

A total of 131 strains of group-A streptococci from 21 of the 22 incidents of skin sepsis (Tables 1, 2) were received for typing. When submitted to the routine typing procedure, all of them fell into one or other of the recognized T-agglutination patterns (Table 3), but only four (3 %) possessed M antigens that were detectable with the antisera then in our possession (see Materials and Methods). The M antigens detected were M33 (in single strains from incidents 3 and 4), M11 (in one strain from incident 13) and M(PT) Truro (in one strain from incident 10). It was thus clear that none of the outbreaks could be attributed to members of recognized M types.

The predominent T-typing patterns of the streptococci were ones frequently encountered among strains from impetigo in children (Parker *et al.* 1955; Wannamaker, 1970). It was rare for all of the strains from one incident to have the same T-typing pattern, but a single pattern tended to predominate in many of the abattoir and factory outbreaks (e.g. nos. 1–3, 5 and 10). On the other hand, streptococci with each of the common T patterns were obtained from a number of different incidents.

When tests for the production of OF had been made, the pattern was somewhat more complicated. For example, among the T25/Imp 19 strains, those from incidents nos. 1 and 5 differed in OF reaction, and thus clearly belonged to different M types; for the same reason, the T3/13/B3264 strains from incident no. 10 included representatives of at least two M types.

*Investigations arising from the first Shropshire outbreak*

The 31 distinct strains from the 1974 Shropshire outbreak comprised 27 with the T-typing pattern 25/Imp 19 (20 from skin lesions; four from the respiratory tract only, and three from environmental sources) and four with the T pattern 5/27/44 (two from skin lesions and two from the throat only, all in the latter part

Table 3. *Results of T typing, initial M typing, and opacity reaction on group A streptococci from incidents of skin sepsis among meat handlers*

| Incident no. | No. of strains typed | Number of strains with T typing pattern (and opacity reaction) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 25/Imp 19 | 14 | 3/13/B3264 | 5/27/44 | 28 | 11 | Other |
| 1 | 31 | 27 (+) | . | . | 4 (+) | . | . | . |
| 2 | 11 | . | 11 (—) | . | . | . | . | . |
| 3 | 18 | . | 17 (−) | 1 (−)* | . | . | . | . |
| 4 | 16 | 3 (+) | 3 (−) | 9 (−)* | . | 1 (+) | . | . |
| 5 | 10 | 10 (−) | . | . | . | . | . | . |
| 6 | 2 | 1 (+) | . | . | . | . | . | 1 (−)‡ |
| 7 | 1 | . | . | 1 (−) | . | . | . | . |
| 9 | 11 | 5 (+) | 1 (−) | . | . | 3 (+) | 2 (+) | . |
| 10 | 8 | . | . | 5 (+), 2 (−) | . | . | . | 1 (+)§ |
| 11 | 5 | . | 5 (−) | . | . | . | . | . |
| 12 | 4 | 1 (−) | . | . | 2 (+) | . | . | 1 (+)¶ |
| 13 | 3 | . | 1 (−) | . | . | 1 (+) | 1 (+)† | . |
| 14 | 2 | . | 1 (−) | . | . | 1 (+) | . | . |
| 15 | 2 | 1 (+) | 1 (−) | . | . | . | . | . |
| 16–22 | 7 | 1 (+), 2 (−) | 1 (−) | 1 (−) | 2 (+) | . | . | . |
| Any | 131 | 38 (+), 13 (−) | 41 (−) | 5 (+), 14 (−) | 8 (+) | 6 (+) | 3 (+) | 2 (+), 1 (−) |

+ = Opacity reaction positive; − = opacity reaction negative.
* One strain M-type 33. † M-type 11. ‡ T-pattern 6.
§ T-pattern 12; provisional M-type Truro. ¶ T-pattern 4.

Table 4. *Activity in indirect bactericidal test and opacity-inhibition test of sera of patients MR and CR against selected Shropshire strains and the type strain of provisional M-type* 3354

| Streptococcal strain | Growth* of streptococci | | | | Inhibition† of opacity by | |
|---|---|---|---|---|---|---|
| | In human serum‡ MR | In human serum‡ CR | In rabbit antiserum‡ (M-type 1) | Without serum | Serum‡ MR | Serum‡ CR |
| No. R74/2015, wound | ± | + + + + | + + + + | + + + + | + | + |
| No. R74/1659, wound (CR) | ± | + + + + | + + + + | + + + + | + | + |
| No. R74/1657, wound | + + + + | + + + + | + + + + | + + + + | − | − |
| No. R74/1658, wound | + + + | + + + | + + + | + + + | − | − |
| No. SF130/13, M-type 1 | + + + + | + + + + | 0 | + + + + | ... | ... |
| No. R68/3354 provisional M-type 3354 | + | + + + | + + + | + + + | ± | − |

..., Not done.

* + + + +, Confluent growth; + + +, semi-confluent growth; +, > 200 colonies. ±, ⩽ 200 colonies; 0, no growth.

† +, Inhibition; ±, partial inhibition; −, no inhibition.

‡ 0·02 ml.

of the outbreak). The T25/Imp 19 strains were all OF-positive, and in routine typing had not given precipitation reactions with antisera for M types 2, 25, 58, 59 and PT 3354, all of which are OF-positive members of the T-typing complex 25/Imp 19; moreover, they had not given an opacity-inhibition reaction with corresponding anti-OF sera of these types, or of PT 3875, for which we possessed an anti-OF but not a precipitating serum.

We therefore sought to characterize the strains by means of opacity-inhibition tests with sera from convalescent patients from the Shropshire outbreak. Five such sera had been obtained; these were tested against OF preparations of the T25/Imp 19 strains, and two of the sera (from patients CR and MR) inhibited opacity production by 23 of the strains but not by the remaining four. This suggested that two different M types were present, the less common having been isolated from skin lesions in four patients and the more common from the remaining sites.

It was necessary, however, to confirm that these sera did not contain antibody against any other OF-positive serotype. When screened against OF preparations of the type strains of existing OF-positive M types and provisional types, a weak reaction was obtained between the extract of strain R68/3354, the type strain for PT 3354, and serum MR but not serum CR. It seemed possible, therefore, that this type might be present among the Shropshire strains, but that we had failed to identify it n the earlier tests because our anti-OF serum was not sufficiently potent.

To investigate this, indirect bactericidal tests were set up with the two human sera and the relevant bacterial strains; these comprised (1) four Shropshire T25/Imp 19 strains (two of which had given positive inhibition tests with the human sera and two of which had not), (2) the type strain for PT 3354 and (3) strain SF/130/13, the type strain for M-type 1, as control (Table 4). Serum CR did not have any 'bactericidal' activity against strain R74/1659 and R74/2015, though it had specifically inhibited their OF; serum MR, on the other hand, had bactericidal activity against both of these strains, and against the type strain of PT 3354. It was therefore concluded that the opacity inhibition reaction between serum MR and the type strain of PT 3354 could be attributed to the additional presence in the serum of M antibody against PT 3354, presumably as a result of an earlier infection, but that the Shropshire strains were distinct from PT 3354.

Having concluded that two distinct T25/Imp 19 strains were present in Shropshire, attempts were made to prepare rabbit antisera against them. Strain R74/2015 was taken as a representative of the strain for which patients MR and CR possessed neutralizing antibody. An antiserum with precipitating, anti-OF, and 'bactericidal' activity was prepared with little difficulty. With strain R74/1658, a representative of the second Shropshire T25/Imp 19 type, an anti-OF serum with specific 'bactericidal' properties has been prepared; further attempts to produce a precipitating antiserum are in progress.

On the strength of these results, we felt justified in recognizing two new provisional M types PT 2015 and PT 1658, and the rabbit antisera were used to screen OF-positive strains with the T-typing pattern 25/Imp 19 from the other incidents among meat handlers (nos. 4, 6, 9, 15 and 22). No representatives of PT 2015 or PT 1658 were found.

*Investigations arising from the second Shropshire outbreak*

In the second Shropshire outbreak (incident 2), in November 1975, all the streptococcal strains reacted with the T-antiserum 14 and were OF negative. A few weeks before, the Kent outbreak (incident 3) had occurred, and this proved to be due to a similar strain. The only previously recognized M types with this T-antigen were M14 (OF negative) and M49 (OF positive), and the Shropshire and Kent strains belonged to neither of these; and we had not previously encountered many OF-negative T14 strains that did not belong to M-type 14. It therefore seemed justifiable to attempt to make rabbit precipitating antisera with randomly selected T14 strains from Shropshire, particularly because OF-negative strains usually give a reliable anti-M response in rabbits. Two strains were selected, one for the earlier part of the outbreak (R75/2681) and one from the end (R76/639). Good precipitating antisera, with corresponding 'bactericidal' activity, were obtained with both.

These sera were used to screen T14 strains from Shropshire, Kent and elsewhere, and every one of them reacted with both the sera. To our great surprise, therefore, we had identified a provisional M type (2681) among the strains from meat handlers that appeared to be very widespread in distribution, having been isolated in nine

Table 5. *Frequency of provisional type (PT)* 2681 *among strains from meat handlers and other sources received for typing between January* 1974 *and June* 1976

| Source | Total no. typed (1) | No. (and percentage) with characters T14, OF – * (2) | No. of (2) tested with PT 2681 antiserum (3) | No. (and percentage) of (3) giving a positive reaction (4) |
|---|---|---|---|---|
| Britain; meat workers | 179 | 44 (25) | 44 | 44 (100) |
| Britain; all other sources | 2816 | 26 (1) | 20 | 19 (95) |
| Other countries | 615 | 66 (11) | 39 | 6 (15) |

* T-agglutination pattern 14; opacity-factor negative.

geographically distinct places in England, and accounted for 31 % of all streptococci from this source.

*Occurrence of provisional M type* 2681 *among streptococci not from meat handlers*

Retrospective investigation was made of the distribution of PT 2681 among other group-A streptococci submitted for typing between January 1974 and June 1976 (Table 5). In this period, we had typed 2995 cultures from Britain, including 179 from meat workers; the latter included, in addition to the 131 previously considered, a number of replicate strains and a few others from the early months of 1974. Of the 2816 cultures not stated to have come from meat workers, only 26 (< 1 %) were OF-negative T14 strains; 19 of the 20 of these that were available for re-examination belonged to PT 2681.

During the same period of time we had examined 615 strains from foreign sources, and a considerably larger number of these (66; 11 %) were OF-negative T14 strains. Of the 39 that had been kept, only six (15 %) belonged to PT 2681. The foreign strains nearly all formed part of four large collections, three mainly from children, in northern Queensland, Bangkok and Trinidad (all three including a high proportion of skin streptococci) and a miscellaneous collection of strains from hospital patients in Homburg, Germany. Although OF-negative T14 strains comprised over 10 % of the Australian and Thai isolates, none of the 34 tested belonged to PT 2681; the Trinidadian collection included few OF-negative T14 and no PT 2681 strains. On the other hand, six of the 11 OF-negative T14 strains from Germany were of PT 2681.

## DISCUSSION

As a result of the present investigation, our ability to M type the group-A streptococci at present causing skin sepsis among meat handlers improved dramatically (Table 6). Initially, we had been able to identify the M antigen of only four of 131 strains (3 %), three as members of internationally accepted types (M11 and M33) and one of a provisional type (PT Truro) that we had earlier recognized as a cause of impetigo among patients in a mental hospital (G. I. Barrow, W. R. Maxted and C. A. M. Fraser, unpublished). By T typing nine

Table 6. *Final results of T and M typing of group-A streptococci from meat handlers*

| T-typing pattern | M type or provisional type | OF reaction | Number of strains | Number of incidents of infection |
|---|---|---|---|---|
| 25/Imp 19 | PT 2015 | + | 23 | 1 |
| 25/Imp 19 | PT 1658 | + | 4 | 1 |
| 25/Imp 19 | ... | + | 11 | 5 |
| 25/Imp 19 | ... | – | 13 | 3 |
| 14 | PT 2681 | – | 41 | 9 |
| 3/13/B3264 | ... | + | 5 | 1 |
| 3/13/B3264 | 33 | – | 2 | 2 |
| 3/13/B3264 | ... | – | 12 | 4 |
| 5/27/44 | ... | + | 8 | 3 |
| 28 | ... | + | 6 | 4 |
| 11 | 11 | + | 1 | 1 |
| 11 | ... | + | 2 | 1 |
| 4 | ... | + | 1 | 1 |
| 6 | ... | – | 1 | 1 |
| 12 | PT Truro | + | 1 | 1 |

..., Not determined.

different T patterns were seen, and two of the more common of these could be further subdivided into OF-positive and OF-negative strains.

Intensive study of the strains from the two Shropshire outbreaks led to the identification of three new provisional types (PT 2015, PT 1658 and PT 2681), and made it possible to M-type 55 % af all strains from meat workers. Two of these types (PT 2015 and PT 1658) were found only in one abattoir (incident 1) and strains with the same T-typing pattern isolated from meat workers in other parts of the country were clearly distinct from both of these. The third new provisional type (PT 2681), on the other hand, was responsible for infections in nine geographically distinct areas of the country, and comprised 31 % of all strains from meat handlers. It was rarely found (< 1 %) among group-A streptococci from other sources in Britain. Although apparently present elsewhere in Europe, it was not encountered among collections of streptococci from skin lesions in parts of central America, Asia and Australia.

Our results confirm that many of the outbreaks of skin sepsis in meat-handling establishments are associated with the wide dissemination of certain strains of group-A streptococci among the workers. Handlers of meat are obviously at risk for sepsis because of the frequency with which they suffer trauma to the hands, and their working conditions provide numerous opportunities for the spread of potential pathogens by indirect contact. Indeed, there was evidence of deficient provisions for hygiene – particularly in the washrooms – in several of the meat-handling establishments. Whether more specific predisposing factors operate in meat-handling establishments is not yet certain. Our colleagues, Dr R. J. Gilbert and Miss Antonnette Wieneke, in the Food Hygiene Laboratory, performed experiments with three strains of group-A streptococci from meat handlers, including

two from the first Shropshire outbreak, and demonstrated a 4- to 75-fold increase in numbers, from an initial inoculum of $10^4$–$10^5$ colony-forming units per g, in raw minced meat kept at 22 °C for 24 h. If similar increases occur in meat-processing establishments, or in the environment of abattoirs, the risk of infection might be increased.

Although many different serotypes of group-A streptococci are at times associated with sepsis in meat handlers, a single serotype (PT 2681) was widely disseminated among them in many parts of the country but appeared to be rare elsewhere in the community. This raises the possibility that some of the infections might have an even more specific association with contact with raw meat, either by virtue of the ability of certain streptococci to survive longer or multiply more freely in meat than others, or because the streptococci have an animal host. The latter possibility may seem remote; the isolation of group-A streptococci from animal sources is rarely reported (Ginsberg, 1972), but one serotype (M50) is specifically associated with natural disease in the laboratory mouse (Hook, Wagner & Lancefield, 1960). Further investigations of these possibilities are needed.

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