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Some Characteristics of Nontypable Group A Streptococci*

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INTRODUCTION

M protein is well established as a determinant of virulence in human infections due to the group A hemolytic streptococcus and as the stimulus for the production of type-specific M protein antibodies(1). However, there are numerous instances documented(2–5) and undocumented, of infections as well as cases of rheumatic fever and nephritis in which strains of group A hemolytic streptococci which could not be typed serologically with the available typing sera were isolated.

The nontypability[1] of virulent streptococci recovered from patients with streptococcal infections or asymptomatic carriers, needs clarification. Perhaps the most important question concerns the acquisition of immunity. If some virulent strains do not contain M protein, what are the implications in regard to protection against subsequent reinfection by the same strain? A first step is to determine whether or not nontypable strains of group A streptococci cultured from infected individuals or carriers contain M protein.

This report describes current efforts to determine whether nontypable strains of group A hemolytic streptococci recovered from individuals with symptomatic streptococcal infections possess M protein and whether other biological properties of these streptococci, such as colonial morphology, hyaluronic acid production, and enzyme production, are related to their virulence.

METHODS

Selection of subjects. Four hundred and ninety-seven patients with suspected streptococcal infections of the upper respiratory tract were screened and 1758

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[1] The term "nontypable" indicates that a particular strain can not be typed with the available typing sera and not necessarily that it lacks M protein.

throat cultures were obtained and processed. From these, 53 strains of group A hemolytic streptococci, selected for investigation, were recovered from patients who had characteristic and unequivocal clinical and bacteriologic evidene of a streptococcal infection. The diagnosis was based primarily on clinical and bacteriologic findings. It is difficult to differentiate some viral upper respiratory infections from streptococcal infections but the clinical and bacteriologic finding in these 53 cases which strongly favored the diagnosis of pharyngitis or pharyngotonsillitis of streptococcal etiology, were their throat cultures on blood agar plates contained 50 or more colonies of group A streptococci, a marked leukocytosis was present in 22 of 23 children on whom leukocyte counts were done, eight patients had scarlet fever, the remainder had pharyngotonsillitis or pharyngitis. Although a significant increase in antistreptolysin and/or antihyaluronidase occurred in only 44.4% of 31 of the patients, the fact that they were all treated early in the course of infection with penicillin probably inhibited production of these antibodies as well as M protein antibody.

Serological grouping and M and T typing. Grouping and typing procedures were those of Swift *et al.*(6). The Todd-Hewitt broth (Difco), in which streptococci were cultured in preparation for typing, contained 2% neopeptone to inhibit proteinase production. T typing was done at the National Communicable Disease Center (N.C.D.C.) in Atlanta.

Selection of strains of group A hemolytic streptococci. When it was determined that a particular strain was group A and not M typable with the available typing sera, five colonies were selected at random from the original culture (10% sheep's blood in agar) and cultured in separate flasks containing 250 ml of Todd-Hewitt broth. These five strains were then subjected to the typing procedure. Selection of five colonies minimized the chance of missing a typable strain.

Selection of group A streptococci rich in M protein. Using a technique which involves resistance to phagocytosis(7), an attempt was made with each strain to select streptococci relatively rich in M protein from those poor in M protein. Streptococci from the same five colonies selected above were treated by this technique. When a test revealed a strain which resisted phagocytosis more than the others, this strain was selected for further study, otherwise any one of the five was subcultured, lyophilized, and stored at room temperature until needed for subsequent tests. Strains selected in this manner were sent to the N.C.D.C. where further attempts were made to establish M types,[2] and T typing was done.

Mouse virulence. Each strain was passed through Albino Swiss mice up to 30 times in an attempt to enhance virulence and increase the likelihood of detection of M protein in strains which may have been capable of M production but not producing detectable amounts. Evidence for increased virulence was determined by the dilution of 0.5 ml of Todd-Hewitt broth culture incubated 18–22 hr at 37°C, necessary to cause severe illness or death of the mouse in 24 hr. A strain, undiluted, which caused no effects with 24 hr before mouse passage, but

[2] Typing sera were available throughout the study in our laboratory for types 1, 3, 5, 6, 11, 12, 14, 15, 17, 18, 19, 23, 24, 27, 29, 31, 32, 36, 37, 38, 39, 43, 46, 47, and 51. Typing sera were only occasionally available for types 2, 4, 8, 22, 25, 26, 28, and 33. Fifty-three strains of group A which could not be typed by us or the N.C.D.C. were the ones on which this study was based.

caused severe morbidity or mortality in a 1:2 or greater dilution after mouse passage, was considered to have increased virulence for mice. The biological tests were performed on each strain of streptococcus before and after five mouse passages. Respective tests of strains were performed in the laboratory chronologically as closly as possible.

Colonial morphology. Colonial characteristics were classified as glossy, matt, or mucoid at the time of initial isolation from the human subject and after each series of five mouse passes.

Capsules. The presence or absence of capsules were determined before and after mouse passage by the India ink method which proved to be relatively crude.

Hyaluronic acid. The presence or absence of hyaluronic acid in the growth medium (brain–heart infusion broth) was determined by Seastone's method(8).

Hyaluronidase. The method for determination of hyaluronidase was based on a modification of the mucin clot-prevention technique(9).

Streptolysin O and S. Each strain of hemolytic streptococcus was tested quantitatively for streptolysin O and S production before and after mouse passage using the method of Hardin *et al.*(5) for streptolysin O, and that adapted by Bernheimer and Rodbard(10) for streptolysin S.

Proteinase. Tests for this enzyme by the method of Elliott and Dole(11) were carried out before and after mouse passage.

Erythrogenic toxin. Each strain of streptococcus was tested in rabbits for erythrogenic toxin production before and after mouse passage using the methods of Wadsworth(12) and Smith(13).

Effects on tissue cell lines. To determine whether an increase in mouse virulence after mouse passage might be associated with increased pathogenicity for human tissue cells, three human cell lines—human ectoderm (conjunctiva-Chang), entoderm (liver-Chang), and mesoderm (heart-Girardi)—were challenged with each strain of streptococcus before and after five mouse passages. The technique used(14) made it possible to observe the tissue cells for cytopathogenic effects which were graded as: (1) total destruction; (2) subtotal destruction; (3) all cells damaged but not destroyed; (4) subtotal damage of some cells; and (5) no cytopathic effects. The cytopathic effects, observed at 24 hr after inoculation with the streptococci and 48 hr after inoculation, were graded according to this arbitrary scale. By comparing the grades, it could be determined whether a particular strain had changed in its ability to cause cytopathic effects on any of the three cell lines before and after mouse passage. The criteria for cytopathic effect were those described previously(14).

M-like antibody production in rabbits. One reason why some strains of group A hemolytic streptococci can not be typed serologically is that they may belong to a type for which typing serum is not available. It is possible that rabbits inoculated with nontypable group A streptococci might produce specific M protein antibodies against the homologous "immunizing" strain, thereby furnishing evidence that the immunizing strain did have M protein even though it could not be typed with available typing sera. Accordingly, rabbits were inoculated with the nontypable strain of group A streptococci before and after mouse passage—one rabbit for the prepassage strain and one for the postpassage, follow-

TABLE 1
STRAINS OF GROUP A STREPTOCOCCI WHICH BECAME TYPABLE AFTER MOUSE PASSAGE

Number of strains	Type	Number of passes to become typable
7	22	30
1	22	15
1	41	30
1	13	15
1	53	15
1	8	30
1	22	20
2	22	15
1	8	20
1	22	10

ing the schedule outlined by Hayashi and Walsh(15). The rabbits were bled at 4, 6, and 8 weeks after the start of inoculation, and each serum tested for M antibodies. The presence of a precipitate after mixing the rabbit antiserum with an acid extract of the immunizing strain, furnished evidence for the presence of M protein in the immunizing strains of streptococci.

M-like antibody production in patients. When available, convalescent sera collected 6–8 weeks after onset of a positive throat culture were tested by the bacteriostatic test(16) for type-specific M protein antibodies.

Lysogeny. Three indicator strains of group A hemolytic streptococci designated T1, T25, and S43, were used.[3] A single colony of the strain to be tested was picked from a blood agar plate and incubated in brain–heart infusion broth at 30°C for 18 hr. Two-tenths milliliter of this broth culture was then mixed with a heavy inoculum of the indicator strain in a soft agar overlay on hard agar. The plate was incubated at 37°C and observed the next day for plaque formation indicated by clear areas 2–5 mm in diameter where there was no growth due to lysis of bacteria by phage.

RESULTS

M-types. Thirty-six of the 53 original strains remained nontypable after 30 mouse passages. Seventeen became typable with the available typing sera after 10–30 mouse passes, 12 type 22s, two type 8s, and one each of type 13, 41, and 53. Nine became typable after 30 passes, two after 20 passes, five after 15 passes, and one after 10 passes (Table 1).

T-types. T-types were determined for 53 strains before, and for 36, after mouse passage (Table 2). Because of the practice of pooling some of the T-typing sera, it was possible to identify individual strains as one of the T-types contained in the pooled sera but not the specific type. Seven could not be T-typed after

[3] The indicator strains were obtained from Dr. H. D. Slade, Northwestern University Medical School, Chicago, IL.

TABLE 2
T TYPES ISOLATED FROM NONTYPABLE GROUP A STREPTOCOCCI BEFORE MOUSE PASSAGE

T types	Number	T types	Number
12	10	12/44	1
3/13/B3264	5	9/11	1
3/13	4	4/28	1
25/Imp. 19	4	11/12	1
28	4	19	1
11	2	6	1
5/11/12/27/44	4	9	1
8/25/Imp. 19	2	Imp. 19	1
1 14	3	5/27/44	1
3	2	Nontypable	3
5/12/44	1		
Total	41		12

mouse passage, but the remainder belonged to T-type pools containing the same T-types as before passage. Eight belonged to T-types characteristic of skin isolates of group A streptococci(17–19), five 3/13/B3264, two 8/25/Imp 19, and one 5/27/44.

Mouse virulence. Nine strains showed no virulence for mice, that is, the mice were not affected by intraperitoneal inoculation of these nontypable strains of group A before or after 30 mouse passages. Among these were two which were shown to be type 22 and one type 13; the remaining six remained nontypable. None of the strains had any noticeable effect on mice before they had been passed, but after 30 passes, 44 strains increased in mouse virulence. The intraperitioneal inoculum required for the lethal effect of the 0.5 ml inoculum ranged from a 1:2 to 1:16,384 dilution of the culture medium. The characteristics of 44 strains which became virulent after mouse passage were compared with nine which remained avirulent, with nonsignificant differences with the exception that none of the "avirulent" strains produced streptolysin S.

Colonial morphology. Twenty-nine of the original isolates had mucoid colonies. Twelve changed from matt to mucoid during mouse passage, three changed from glossy to mucoid, five remained matt, and four changed from mucoid to matt. Seven of the 29 original isolates, which had mucoid colonies, did not increase in virulence during mouse passage. All 12 strains, with colonial characteristics changing from matt to mucoid, increased in virulence. Five isolates, which remained matt, and four, which changed from mucoid to matt, increased in mouse virulence. These results indicate there was little correlation between colonial morphology and mouse virulence among those 53 strains of nontypable group A streptococci.

Hyaluronic acid. Presumably, the presence of hyaluronic acid in the growth medium originated from the capsules of the streptococci since the capsules contain hyaluronic acid. In 18 strains hyaluronic acid was present in the growth medium after 30 mouse passes, but not before; in three it was present before and after mouse passage; in one it disappeared after mouse passage, and in 31 no hyaluronic acid could be detected before or after mouse passage.

TABLE 3
STREPTOCOCCAL ENZYME PRODUCTION BEFORE AND AFTER MOUSE PASSAGE

	Hyaluronidase	*Streptolysin O*	*Streptolysin S*	*Proteinase*
Number of strains producing	51	53	50	51
Increased production after mouse passage	27	30 (2.2 tubes)	15	22
Decreased production after mouse passage	21	16 (1.7 tubes)	25	20
No change in production after mouse passage	2	7	9	9
Lowest dilution of culture medium	1:2	1:2	1:10	1:10
Highest dilution of culture medium	1:4096	1:256	1:10,000	1:10,000
No production	3	2	3	2

Hyaluronidase. Hyaluronidase was produced by 51 of the 53 strains; in 27 it increased after mouse passage; there was a decrease in 21, and no change in two (Table 3). This enzyme was detectable in dilutions of culture medium ranging from 1:2 to 1:4096. Of the 21 strains which contained hyaluronic acid in the growth medium after mouse passage, eight produced no hyaluronidase after passage.

Streptolysin O. All 53 strains produced streptolysin O. After mouse passage, production increased in 30 strains, decreased in 16, and remained the same in 7 (Table 3). The average increase was 2.2 dilution (tubes). The average decrease was 1.7 dilutions. Streptolysin O production could not be demonstrated in two strains after mouse passage.

Streptolysin S. Streptolysin S was produced by 50 strains. Production after mouse passage increased in 15 strains by an average dilution of 1:3293 and decreased in 25 strains by an average of 1:2468. In nine strains there was no quantitative change in streptolysin S production after mouse passage, and in three none could be detected before or after passage (Table 3).

Proteinase. Proteinase was produced by 51 strains. Quantitative production of proteinase increased in 22 strains after 30 mouse passages; in 20 there was a decrease; no change in nine, and no proteinase could be detected before or after mouse passage in two (Table 3).

Erythrogenic toxin. Erythrogenic toxin was produced by 31 strains. The amount of erythrogenic toxin increased in seven after mouse passage and remained the same in 24. In 11 no erythrogenic toxin could be detected. No results were available for 11.

Plaque formation due to phage. All strains of streptococci were tested for plaque formation before mouse passage. The result (Table 4) showed plaque formation by 45 of the 53, 25 with indicator strain T1, 28 with indicator strain T25, and 22 with indicator strain S43. Five strains showed plaque formation with all three indicator strains, 13 with T1 and T25, five with T1 and S43, and seven with T25 and S43.

Erythrogenic toxin production and lysogeny of streptococci etiologic for scarlet fever. Eight of the 53 patients with streptococcal infections, for whom nontypable group A streptococci were considered etiologic, had scarlet fever. When tested in rabbits, six of the eight strains were shown to produce erythrogenic toxin. All eight showed lysogeny with one or more of the indicator strains. Two children with scarlet fever were infected by group A, nontypable streptococci in which erythrogenic toxin production could not be demonstrated.

M-like antibody production in rabbits. According to the schedule outlined in Methods, nontypable group A streptococci were inoculated into rabbits before and after 30 mouse passes. Seventeen strains became typable during the course of mouse passage, and these were not used to inoculate rabbits because they had shown the ability to produce M protein. After 30 mouse passes, 18 of the remaining 36 strains stimulated antibody production against proteins extracted by the method used to obtain M protein(6), from the homologous immunizing strain. These precipitins were removed by absorption with the immunizing strains, affirming specificity. Twelve strains did not induce antibody of this type before or after mouse passage, in other words, they were devoid of M protein antibody-stimulating capacity. Two strains were antigenic for precipitating antibodies before and after mouse passage, four were antigenic before but not after mouse passage. Nine rabbit sera were tested for the presence of precipitating antibodies against 14 strains used to immunize rabbits. Three strains were considered to possess the same precipitating antibodies since each serum reacted with an acid extract from these three and the precipitins could be removed by absorption with each strain. Since M protein is considered to be associated with virulence, 16 strains which became positive for M-like protein were compared with the 12 which remained negative, for colonial characteristics, production of hyaluronic acid, streptolysin O and S, erythrogenic toxin, proteinase, and virulence. No significant differences were noted in any of these characteristics.

M-like antibody production in patients. Convalescent sera were obtained from 33 patients. There was clear evidence of bacteriostatic antibodies against the homologous infecting strain in five convalescent sera and in one acute-phase specimen; in one, there were antibodies in both the acute and convalescent sera. No bacteriostatic antibodies were found in the remaining 26 patients for whom convalescent serum was available. Two strains, even though nontypable, were able to stimulate M protein-like antibody production in both patients and rabbits.

Effects on tissue cell lines. As might be expected, cells in contact with streptococci exhibited greater tissue cell damage after 48 hr incubation than after 24 hr. The most significant biologic result was the greater cytopathic effect on heart cells when compared to conjunctiva and liver cell lines. Damage was greatest for heart cells in all categories, pre and post mouse passage, and after 24- and 48-hr incubation. All cell lines incubated with streptococci showed slightly more damage by streptococci after they had been passed in mice, but the differences were so slight it is doubtful whether they were of biological significance.

TABLE 4
NUMBER OF STRAINS SHOWING LYSOGENY

Indicator strains	Number showing lysogency with each indicator strain	Number showing lysogency with combinations of indicator strains
T1	25	
T25	28	
S43	22	
T1, T25, S43		5
T1, T25		13
T1, S43		5
T25, S43		7

DISCUSSION

During the past 15 years the majority of group A streptococci recovered from Nashville children, who were carriers or had manifest streptococcal disease, could not be M-typed. Many of these non-typable strains probably do not possess M protein, and therefore could not stimulate M protein antibody production with resulting subsequent immunity although nontypability of a strain when isolated does not necessarily mean it had not produced M protein *in vivo* and induced antibody formation. At present there is no way of knowing if there might be different strains among the nontypables, each with its own separate pathogenicity, but one fact remains certain, that most of the infections in the Nashville study group were caused by nontypable strains. It is possible that some of the children in this study had upper respiratory infections due to some other etiologic agent at the time the group A streptococci were recovered; however, clinical and bacteriologic evidence favored streptococcal etiology. Thirty-six of 53 strains of nontypable group A hemolytic streptococci etiologic for upper respiratory infections and scarlet fever could not be M typed. Immunity to the group A hemolytic streptococcus is considered to be in large part due to antibodies against M protein which are specific for each separate type. Since there is no evidence that type-specific immunity to most of these nontypable strains is acquired, this might be one explanation why they remain endemic in the community. It suggests that there may be other mechanisms of immunity in addition to the acquisition of M protein antibodies against specific types of group A.

The percentage of group A streptococci which can be typed appears to be becoming less. In a study of children with streptococcal pharyngitis in Chicago(2) only 52 % of the group A strains were typable. A third of strains of group A recovered during a study of school children in Nashville, TN between 1953 and 1958, could be typed serologically(20). More recent studies in Nashville school children produced typable group A streptococci in 17% of all isolates(21) and in current studies approximately 11% of all group As were typable (Quinn, R. W. and Federspiel, C. F. The natural occurrence of the hemolytic streptococcus

in children in Nashville, Tennessee. In press, Amer. J. Epidemiol.). These findings were similar to those noted in Memphis, TN between September 1965 and August 1968 where only 13% of group A streptococci from children with sore throats were typable(22). This is a markedly different situation than that found in U.S. Army Air Force military populations of 1951 where 92% of the group A strains were typable. In the U.S. Navy training centers during World War II, the great epidemic of infections due to the hemolytic streptococcus(23) followed by rheumatic fever was caused by group A streptococci, the majority of which were typable, the most common types being 17, 19, and 3. Nevertheless, a significant percentage (16.1%) of nontypable strains of group A were isolated in Naval training centers in 1944(24). An even higher percentage (45.9) of nontypable strains was recovered from recruits with acute respiratory disease at the U.S. Naval Training Center at Great Lakes, Il during January–May 1950(25). Somewhat different was the finding that nontypable strains were carried by about a fourth of carriers in a Massachusetts family study in 1925(26).

The epidemics of streptococcal infections during World War II in the U.S. Navy Training Centers took place in a situation in which susceptible young men from widely spread communities were introduced into an environment inhabited by individuals infected by and carrying virulent, typable strains of group A; the essentials for an epidemic were present. Streptococcal infections in Nashville appear to be endemic with some seasonal fluctuation, but for the past decade and a half nothing resembling a community-wide epidemic has occurred. Contact with children other than those at home, in their own neighborhood, and school was probably minimal in Nashville.

After passing nontypable strains of group A through mice, 17 of 53 became typable indicating that some of the so-called nontypable group A streptococci did posses M-like protein which increased during mouse passage to a level which was detected by the precipitin method. Acquisition of M-like protein was always accompanied by an increase in mouse virulence, but virulence also increased in most strains which remained nontypable. It is significant that none of the nontypable strains at the time of isolation from the patient were virulent for mice, but 44 of the 53 later increased in mouse virulence. This would suggest that virulence for humans and virulence for mice are two very different characteristics. Mouse passage did not always result in an increase in mouse virulence since nine of the 53 strains showed no change. These findings are reminiscent of those of Todd and Lancefield who found the behavior of matt attenuated cultures undergoing mouse passage was frequently capricious so far as virulence is concerned.(27).

Mouse passage stimulated hyaluronic acid production in 33.9%. These results are in striking contrast to those of Seastone(8), who found that about 94% of strains from patients with moderate to severe streptococcal infections produced mucoid polysaccharide, and to those of Hardin *et al.*(5), who found that 43 of 49 strains of nontypable group A streptococci produced hyaluronic acid. The reltively low numbers of hyaluronic acid producers among the 53 nontypable strains suggests the possibility that these strains of streptococci were not as virulent as those studied by Seastone or Hardin. The production of hyaluroni-

dase by some strains, in which hyaluronic acid was also found, would be possible if hyaluronidase were present in minute amounts or if tests for hyaluronic acid were made before hyaluronidase production began to any appreciable extent. To maximize chances of detecting hyaluronic acid, tests for hyaluronic acid were made after 18–22 hr of incubation and those for hyaluronidase after 36 hr. The presence of hyaluronidase of easily detectable amounts in 18-hr cultures is uncommon. Willoughby *et al.*(28) showed that hyaluronate could be detected after 9-hr incubation of group A streptococci. Therefore, it seems likely that hyaluronic acid, had it been present, would have been detected, because hyaluronidase would not have been present in 18–22 hr in amounts sufficient to destroy hyaluronic acid.

The effect of mouse passage on streptolysin O and S production was not consistent, since both increases and decreases in the quantity produced were seen after passage. An increase in streptolysin O was seen twice as frequently as for streptolysin S, and a decrease more often for streptolysin S. If streptolysin O were used as a measure of virulence, it would appear that the strains were more virulent after mouse passage, whereas if streptolysin S were used as the indicator, virulence was decreased after mouse passage. However, none of the strains which remained avirulent after passage produced streptolysin S. These observations are at variance with the concept that virulent forms of beta hemolytic streptococci produce less streptolysin O and S than avirulent cultures(29).

Although many typable strains, which produce M protein also produce proteinase, in a previous study(5) nontypable streptococci produced proteinase four times as frequently as those which were typable. It is significant that all but two of the 53 nontypable strains were shown to produce proteinase, either before or after mouse passage. Neopeptone in the Todd-Hewitt broth, in which the streptococci were cultured before typing would have inhibited proteinase production; therefore, it is unlikely that proteinase would have interfered with typing of these strains.

Plaque formation indicative of lysogeny was relatively frequent, but perhaps even more significant was the demonstration of lysogeny in all of the strains recovered from children with scarlet fever even though in two, erythrogenic toxin could not be demonstrated. The relation between phage and erythrogenic toxin production is under study currently and will be reported later.

Some strains of group A streptococci are nontypable because typing serum is unavailable, either because it is in short supply or has not been prepared for a particular type by immunizing rabbits. When rabbits were inoculated with 36 nontypable strains to stimulate M protein antibody production, 18 of these 36 stimulated antibody production which appeared to be characteristic of M protein antibodies, although they did not belong to any of the known types. Passage through mice enhanced antigenicity of M-like protein for some strains, but this procedure was not always necessary, because six strains stimulated M-like protein antibody production before mouse passage. Among these Nashville strains, three appeared to be the same serological type based on the standard typing procedure. The illnesses caused by the strains which became typable were indistinguishable from those caused by nontypable streptococci, indicating that virulence in these

patients was not significantly different for typable or nontypable strains. There is some evidence that nontypable strains of streptococci can stimulate M-like protein antibody production in patients(3). The impression was confirmed by the finding of presumably M protein antibodies, against the homologous infecting strain in six subjects, suggesting that in some individuals M protein antibodies are produced during infection due to a nontypable strain. These findings also indicate that for the particular strains studied, rabbits were stimulated to produce antibodies against more strains than were humans. To establish these nontypable strains as new types, as done periodically, was outside the scope of this study(30,31).

The greater cytopathic effect of streptococci on heart tissue cell lines (mesoderm) compared with cell lines derived from entoderm or ectoderm, which had been noted previously(14), was confirmed.

SUMMARY

This study confirms numerous observations that nontypable group A streptococci are able to cause streptococcal disease in spite of the finding that the majority apparently did not possess M protein. Some so-called nontypable strains of group A streptococci belong to known types since 17 of 53 became typable during the course of mouse passage. Of the remaining 36, 18 were able to stimulate M protein antibody production, but significantly, 12 remained M negative. The only evidence suggesting that the nontypable streptococci were less virulent for humans than those of 15 years ago, was that significantly fewer strains produced hyaluronic acid. Greater damage by group A streptococci to heart tissue cells (mesoderm) than to cells from other primary cell types (entoderm or ectoderm) was confirmed. Increase or decrease in enzyme production could not be related consistantly to virulence or typability. Virulence for humans and mice appeared to be different properties.

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