TYPES OF HAEMOLYTIC STREPTOCOCCI IN RELATION TO SCARLET FEVER.

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SEROLOGICAL CLASSIFICATION OF SCARLATINAL STREPTOCOCCI.

Material Investigated.

The majority of the strains of haemolytic streptococci of scarlatinal origin were derived from the throats of scarlet fever cases; 34 were obtained from Liverpool, 18 from Manchester and 29 from London. I have also had for comparison two strains, 55/4 and 45/2, from Dr Zingher of the New York Health Department and a Dochez strain; the last was kindly given to me by Dr R. A. O’Brien from the Wellcome Research Laboratories, together with two strains, S.F. 18 and S.F. 22, isolated by Dr Okell. For the Liverpool and Manchester strains I have to thank Dr G. R. James and Professor W. W. C. Topley. Most of the London strains of streptococci I was able through the kindness of Dr Foord Caiger to obtain myself from throat swabs taken from scarlet fever patients by Dr Ruby Inkster at the South-Western Fever Hospital. For two other strains of interest, one from the throat of a nurse with scarlet fever and the second from the cerebro-spinal fluid of a case of meningitis following scarlet fever, I am indebted to Dr L. Colebrook and Dr J. G. Forbes respectively.

Preliminary Observations.

In agglutination work with streptococci one is met with the difficulty of making stable suspensions. This difficulty, which has been the general experience, has been overcome with more or less success by different investigators in various ways. I wished, if possible, to use for agglutination whole cultures in broth without resorting to devices for re-suspending cultures which ordinarily deposited during growth. The majority, however, of my strains produced in broth either a granular deposit or an unstable culture which flocculated spontaneously on being heated in the water-bath at 55° C. To these cultures the ordinary method of agglutination was not applicable as a routine procedure, but I have found a rapid method of agglutination (to be described below) useful for orientation purposes.

The chief aim of this study of haemolytic streptococci was in the first place to define serological types among the scarlatinal group and ultimately to determine the distribution of such types among streptococci from other sources. For this classification the final criterion is the test for absorption of agglutinin. In this test stable suspensions of the homologous strains are
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necessary for trial of the effect of absorption, and it has happened that the more important strains from which sera were prepared produced with fair regularity uniform growths in broth (reaction pH 7-8) prepared with trypsinised meat. It is an interesting fact that occasionally a streptococcal strain will grow in such a broth as uniformly as the pneumococcus and will remain permanently stable, while at other times the same strain begins to granulate after standing for 24 hours. I have not been able to form any conclusion as to whether these different characteristics of growth correspond with changes in antigenic properties.

In agglutinin absorption experiments with streptococci it is important to use only a moderate amount of culture for absorption, i.e., very little more than is necessary to exhaust the serum with the strain used in its preparation. Employed in this manner the test picks out strains in which the dominant antigen is the same and yields results upon which a useful classification may be based. If too heavy an absorption dose is used, individual differences between strains are liable to be masked and confusion is caused by partial absorption with non-identical but closely related strains.

I now proceed to describe in detail the technique which I finally adopted.

Preparation of Agglutinating Sera.

As is usual at the outset of an investigation of this character, a number of strains were taken haphazard for the preparation of agglutinating sera. These included Type 1 (S.F. 130, from Manchester), Type 2 (S.F. 22, from the Wellcome Research Laboratories), Type 3 (55/4, from Dr Zingher, U.S.A.); other strains were Dochez, 45/2 (Zingher), S.F. 14, 155 and 73 (Manchester), and S.F. 5 (Liverpool). It was discovered subsequently that, while a single representative of each of the Types 1 and 2 had been selected, no less than three out of the nine strains belonged to Type 3, viz. 55/4, S.F. 14 and S.F. 155.

Adult rabbits were used and the injections were given intravenously. For the first five weeks the rabbits received culture killed by heat and then living culture was given, beginning with 2 c.c. of broth culture subcutaneously. This was followed by two series of intravenous injection in successive weeks, the largest dose being 4 c.c. of broth culture. Several of the rabbits died of septicaemia and living culture was discontinued. The subsequent routine procedure was to inject the deposit of 100 c.c. of heated broth culture every week in two or three doses. In some cases immunisation has been prolonged for five months without a usable serum being obtained, but generally a period of three months is sufficient.

It is often difficult to be certain of the exact limit of the titre of a streptococcal serum, since it varies with the sensitiveness of the suspension; but all the three type sera have agglutinated well above 1 in 1600; the Type 2 serum in use at present agglutinates over 1 in 12,000 with test suspensions insensitive to sera of other types. Since adopting for preliminary diagnosis of type the method of agglutination described subsequently, I do not make a final bleeding
of the rabbit until its serum in a dilution of 1 in 25 agglutinates at once on
a slide a fairly dense suspension of its homologous strain. It has been found
from experience that this reaction indicates that such a serum can be used
for the ordinary method of agglutination in the water-bath.

Agglutination by Rapid Method.

It was observed in the course of the work on absorption that those strains
which removed the agglutinin from a serum agglutinated almost at once
in the 1 in 100 dilution with the formation of coarse clumps. This suggested
that the method of agglutination which consists in mixing on a slide drops of
a powerful agglutinating serum and a fairly dense suspension of culture, and
watching the result under a low magnification, might be applicable to haemo-
lytic streptococci. The use of moderately granular suspensions would then be
possible and the spontaneous flocculating effect of heating in the water-bath
would be avoided. The following procedure was adopted. The culture for test
was grown overnight in a few cubic centimetres of trypsinised meat broth and
was then centrifuged. The supernatant broth was removed with the exception
of a few drops in which the deposit was re-suspended with the aid of a finely
pointed teat pipette. The immune sera to be tested were diluted 1 in 25 and
drops of each diluted serum were placed side by side on a glass slide. A drop of
suspension was then placed close to each drop of serum and the two were mixed
with a platinum loop, the process being watched under a dissecting microscope.

In the case of the three type sera the agglutination results and the absorp-
tion results agreed; each strain which had given a positive absorption result
with a particular serum agglutinated almost at once with the formation of
coarse clumps, while little or no change was observed in the same time with
the heterologous sera. I have found this quick method of agglutination of
considerable value as a preliminary test for the detection of types before
proceeding to the absorption test. A large number of sera can be tested against
one strain in a very short time, and the number of sera against which it is
worth while to make an absorption experiment may be reduced to manage-
able proportions. Certain precautions must be observed with regard to the
culture suspensions. Those with coarse granules cannot be used, but a moderate
degree of granularity does not cause confusion. A more important point is
that some strains acquire rather slowly in culture this property of clumping
rapidly at room temperature and, if young broth cultures are used on account
of their more uniform growth, there may be no trace of agglutination even
with the homologous serum. The same culture allowed to grow for 18 hours
has given the characteristic reaction. On the other hand, some strains, allowed
to grow overnight, become too sensitive to agglutination and there may be
marked clumping with some or all of the heterologous sera. One must recognise
that this method, like the direct agglutination test, has its limitations in the
case of streptococci.
As explained earlier, the purpose of the test of absorption of agglutinin was to select strains with the same dominant antigen and the dose of absorbing culture was an important consideration. With the sera used it was found that the centrifuged deposit of 100 c.c. of an overnight broth culture (not heated) added to the serum to make a total quantity of 1.5 c.c. of 1 in 100 dilution was sufficient to remove the homologous agglutinin. The absorbing strain and serum were left in contact in the refrigerator overnight, and the next day the mixtures were centrifuged until the supernatant fluid was clear. The lowest dilution of absorbed serum tested was 1 in 200 after the addition of the test suspension, and the titration was taken to 1 in 3200.

So soon as the preliminary stages of the investigation revealed the presence of three definite types among haemolytic streptococci of scarlatinal origin, it became the routine to put up every strain under examination against each of the three sera, irrespective of whether agglutination occurred or not. This was a laborious undertaking and, as subsequent events showed, unnecessary tests might be avoided by preliminary direct agglutination. The latter test could not, of course, be done where the strains grew in the form of a granular deposit. In order to ensure that in the absorption test the agglomeration of cocci brought into contact with the agglutinin should be as small as possible, such granular deposits were emulsified in the serum dilution by means of a fine teat pipette the point of which was pressed against the floor of the tube; the mixture was thus forced repeatedly through a narrow chink and the clumps well broken up. The majority of the strains belonging to the three types were readily detected, for strains of the same type generally absorbed the agglutinin as efficiently as the homologous, though sometimes there would be moderate agglutination in the first two tubes of the titration.

Difficulties have, however, been experienced. It has occurred that a strain has failed to absorb on repetition of the test, the treated serum agglutinating the homologous as fully as the control or differing only in producing smaller clumps. Further tests have generally proved that such a strain could take out the dominant agglutinin. In two instances, however, strains once positive remained afterwards consistently negative.

Variations from time to time in the extent of the removal of agglutinin by a particular strain may sometimes be accounted for by differences in the amount of culture used for absorption. The absorbing dose, i.e., the centrifuged deposit of 100 c.c. of broth, has not been estimated exactly and may vary in amount on different occasions. And in this connection it should be remembered that a small amount of agglutinin left in the serum may have a considerable agglutinative effect on a very sensitive streptococcal suspension, particularly one which deposits fine granules on standing.

In an absorption test on an unknown strain it is advisable to put up in parallel several other strains, which should include (a) the homologous, and
(b) a strain or strains which previous tests have proved to be incapable of exhausting the particular serum of its agglutinin.

Classification of Results.

Absorption tests, combined with direct agglutination by the rapid method described, have been made on each of the three type sera with 81 strains of haemolytic streptococci obtained from the throats of scarlet fever patients, and 37 of these strains have fallen into one or other of three types. The types are distributed as follows: 12 of Type 1, 14 of Type 2 and 11 of Type 3; two additional strains agglutinated rapidly both with Type 1 serum and Type 3 serum and absorbed the homologous agglutinin from both sera. The remaining 42 strains conformed to none of the three chief types.

This series of scarlatinal streptococci, therefore, comprises three chief types and a number of unclassified strains.

From the latter a number of strains have been selected and used for the preparation of agglutinating sera in rabbits. Seven have so far yielded sera which gave rapid coarse agglutination by the slide method. The strains appear to possess individual antigens and, in cross-agglutination tests with these seven, typical agglutination occurs only with the homologous strain and serum. The Dochez strain falls amongst the unclassified strains, and, although the majority of strains have been tested against its serum, the only homologues found are the Zingher strain 45/2 and a strain from a fatal case of cerebro-spinal meningitis following scarlet fever.

The unclassified scarlatinal strains apparently form a heterogeneous group.

Relationship between the Three Chief Types of Scarlatinal Streptococci.

The three main types have been defined by a method of absorption where just sufficient culture has been used to exhaust a serum of its homologous agglutinin by means of the strain with which the serum was prepared. Such a test reveals the dominant antigen of a strain, and neglects the subsidiary antigens, which closely related strains might be shown to possess in common, and which would be demonstrated by the ordinary direct agglutination test.

In order to show that the dominant antigens of the three type strains are separate and distinct, I have increased the absorbing dose in cross-absorption experiments to two and three times the usual amount, but I have not attempted to ascertain whether still larger masses of culture will exhaust the heterologous sera. The strains of Types 1 and 2 are very sharply differentiated from each other by absorption, and the ordinary direct agglutination test shows that the dominant antigen of one type is scarcely if at all represented in the other (see Table I).

On the other hand, Types 1 and 3 show evidence of inter-relationship, although, in the particular strains which I have used to prepare type sera, the dominant antigens which differentiate them seem to be fairly pure. Other strains of Type 1 do, however, lower the titre of Type 3 serum, and two strains
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have been obtained which remove the whole of the agglutinin from both Types 1 and 3 sera. These two strains also agglutinate rapidly with both sera. I have plated cultures of both strains and have attempted to separate colonies which might possess one antigen only. From one strain several colonies, differing slightly in appearance from the majority, were obtained which agglutinated rapidly with Type 1 serum only, while the majority still agglutinated with both sera. There was, however, apparently still sufficient of the Type 3 antigen remaining in this new strain to exhaust Type 3 serum of its agglutinin, in spite of the lack of response shown to this serum in the rapid agglutination test.

Table I.

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<th>Strain</th>
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<th>Type 3 serum</th>
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<td>Dochez</td>
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Table I shows the results of agglutination tests with stable suspensions in the water-bath at 55° C. for several hours and after standing overnight at room temperature. There is seen to be, in the dilutions used, no cross-agglutination between Types 1 and 2. In addition, there is no evidence of a common agglutinative factor which would permit of classing these scarlatinal strains into one group. The Type 3 suspension was slightly less stable than the others and threw down some fine granules after standing overnight. There is evidence of relationship between the Types 1 and 3 strains.

Discussion.

The above results show very clearly that among haemolytic streptococci obtained from cases of scarlet fever there are strains which are characterised by perfectly distinct antigens; these are the streptococci of Types 1 and 2. There is a third type, Type 3, which is closely related to Type 1 but appears quite distinct by the method of absorption employed, that is to say, its dominant antigen is different from that in typical strains of Type 1 or of Type 2. This is shown by the fact that some strains, which have been classed as Type 1 because they exhaust Type 1 serum, also partially absorb the serum of Type 3, while there are certain strains which are capable of exhausting both Types 1 and 3 sera.

Types 1 and 2 correspond with types of the same designation discovered in Aberdeen by Dr J. Smith1 with whom I have exchanged cultures and sera.

Smith was able to classify 175 out of 210 strains of scarlatinal streptococci as Types 1 and 2, leaving only 35 unclassified. In my investigation the proportion of Types 1 and 2 strains to the whole is much smaller; the discrepancy may be merely a question of local differences in distribution, but I have considered other possible explanations. A number of my unclassified strains may be examples of Type 1 or Type 2 which have become degraded, either in the throat or in culture, and unable, therefore, to absorb the agglutinin from serum made with an intact strain. Against this view it may be pointed out that certain of the above-mentioned strains, taken haphazard, have produced sera which characterised them as individual types. It might also be argued that, in the case of strains not conforming to any of the three types, the colonies selected from the plates made from the swabs were not representative of the scarlatinal streptococci, but were colonies of non-specific haemolytic streptococci ordinarily found in the throat. This suggestion is difficult to dispose of at the present stage; it would imply that there is, during an attack of scarlet fever, a simultaneous multiplication of specific and non-specific streptococci. In default of evidence of such an occurrence, it is reasonable to assume that at least the majority of the streptococci not conforming to the three types bear the same causal relationship to the scarlet fever in which they were found as those which do conform.

This view is supported by the analogy with pneumococci, where many individualistic strains which have to be classed in the heterogeneous Group IV are undoubtedly responsible for pneumonia.

The existence of serological types of scarlatinal streptococci, which are sharply defined both by agglutination and absorption, is difficult to reconcile with the results of investigators who find that scarlatinal streptococci form a group with common agglutinative characters.

There is the possibility of a series of strains being all of the same absorption type, though I do not think that this is the case in Eagles’s investigation, where the agglutination tables give a definite indication that the sera were made from different types.

I think that, owing to the instability of streptococcal suspensions, there is a tendency for them to react with very small amounts of agglutinin, and thus differences in antigenic structure may fail to be revealed by the ordinary agglutination test in the water-bath. It has been mentioned earlier that by the rapid agglutination method very different results are obtained according as the suspension is stable or hypersensitive; in the latter case a strain gives agglutination with sera made from different types, whilst in the former the reaction may be limited to the homologous serum.

Another possible explanation of discrepancies in the results of different workers on streptococci may be the existence of group and specific phases.

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similar to those discovered by Andrewes in the paratyphoid group of bacteria. I have given some attention to this point and had thought at one time that the relationship between streptococcal Types 1 and 3 might be that of specific to group. In spite of repeated plating of these cultures I have been unable to demonstrate any segregation of these characters.

Comparison with Haemolytic Streptococci not Related to Scarlet Fever.

Puerperal Fever.

Cultures of haemolytic streptococci from 46 cases of puerperal fever have been examined; the majority of these were obtained from the blood, the rest from the uterus or cervix. This series of strains was collected by Dr L. Colebrook, and I am greatly indebted to him for his kindness in giving me subcultures. All the above strains were tested by the rapid method of agglutination and by agglutinin absorption against each of the three type sera. Of the 46 strains, four belonged to Type 1 and one to Type 3 of the scarlatinal group; no representative of Type 2 was found amongst them.

An analysis of the relationship of the remaining 41 puerperal streptococci to each other and to those strains of the scarlatinal group which did not conform to any of the three types has been begun, and, though the examination is incomplete, some points of interest have been made out.

Agglutinating sera have been prepared in rabbits with eight puerperal strains. Six of the sera give rapid coarse clumping with their homologous suspensions, when tested by the slide method of agglutination already referred to.

The results obtained by this rapid method of agglutination have been arranged in the form of a table, together with similar results obtained with scarlatinal strains and their antisera for the purpose of comparison.

Before commenting on Table II, I must explain the significance of the plus and minus signs. The plus sign indicates that, within a few seconds after mixing on a slide the drops of serum (1 in 25) and suspension together and stirring the mixture with a small platinum loop, well-marked clumping occurred. In the case of the minus sign there was within the same period little or no evidence of increase in the size of the particles of the suspension. It is necessary to limit the period of observation, since after 1 or 2 minutes the more sensitive suspensions often begin to show the formation of small flocculi with one or more of the heterologous sera. On repetition of such a test with a younger and more stable suspension this agglutination with heterologous sera has not occurred. It is, therefore, justifiable, I think, to infer that two strains which give a plus reaction with the same serum have the same dominant antigen,

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Table II.

Agglutination by Rapid Method of Scarlatinal and Puerperal Cultures of Streptococci with their Antisera.

Sera diluted 1 in 25.

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<th>Strains*</th>
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* Type 1 to S.F. 13 are scarlatinal and P. 1 to P. 15 puerperal strains.

On examination of the table, it appears that most of the puerperal strains tested are individual strains, two only, P. 1 and P. 4, being of the same type. Two give cross-agglutination with scarlatinal types 1 and 3, with which types they have already been identified by agglutinin-absorption. Similarly, those scarlatinal strains which failed to absorb the three type sera are also highly individual, and the dominant agglutinin of their antisera rarely affects any other strain in either group. One only of these scarlatinal strains and its serum give cross-agglutination with a puerperal strain and its serum.

Further investigation may show that certain types have a particular association with the clinical groups of puerperal strains—the two strains, P. 1 and P. 4, may, perhaps, be the nucleus of such a type—but there is no strong indication at present of this being the case.

Other Infections.

Out of nine strains of haemolytic streptococci from non-scarlatinal throats, Type 1 occurred once; in comparison, five throat swabs, from a school where outbreaks of scarlatina had occurred, though none was in progress at the time of swabbing, yielded Type 2 strains twice and Type 3 once. A case of cerebrospinal meningitis, not known to have been associated with scarlatina, yielded a Type 1 strain from the cerebro-spinal fluid. This series is very small, but it...
has been given because it shows that the type strains can occur in the throat etc. apart from scarlatina.

Discussion.

In the puerperal group the frequency of strains of the three types is very much less than in the scarlatinal group, and it is noteworthy that no representative of the scarlatinal Type 2 was found. The whole group of puerperal strains apparently consists of a number of types (apart from the two chief scarlatinal types) which recur infrequently, and, so far as the results at present go, it resembles in its diversity the collection of unclassified strains in the scarlatinal group.

In the scarlatinal group of streptococci I have shown that rather less than 50 per cent. were identified with the three chief types. The distribution of the types will, no doubt, vary in different localities. In the small series of London cases included in my total, 16 out of 28 strains (= 57.1 per cent.) were typed as follows: 16 collected in April—May, 1925, yielded 4 strains of Type 2 and 5 of Type 3, whilst the remaining 12, collected in January—February, 1926, comprised 3 of Type 1, 2 of Type 3 and two which absorbed both Types 1 and 3 sera.

To recapitulate, if one views as a whole the two clinical groups of haemolytic streptococci, the scarlatinal and the puerperal, it may be considered as a group comprising three well-defined types and a collection of heterogeneous strains. On closer analysis it would be observed that the types occur frequently among the strains of scarlatinal origin and rarely among the puerperal strains, which are chiefly individual strains falling in the heterogeneous collection.

Significance of Streptococci in Scarlatina.

The basis on which has been founded the thesis that scarlatina is due to a specific scarlatinal streptococcus consists of:—(1) the presence of haemolytic streptococci in scarlet throats in over 90 per cent. of acute cases, the percentage in a normal population being rarely more than 10; (2) the production of clinical scarlet fever (a) by cultures of such a haemolytic streptococcus sprayed on a susceptible throat, (b) by injection of the “toxin” produced in broth by the growth of such a streptococcus; (3) the fact that an antiserum prepared with such a toxin (a) will give the Schultz-Charlton reaction, i.e. behaves like the serum of a scarlatinal convalescent towards the scarlet rash, and (b) clinically ameliorates the symptoms of scarlet fever; and (4) the correlation between susceptibility to scarlet fever and sensitiveness (absence of immunity) to the streptococcus toxin—the Dick test. None of these is convincing alone, and even in the aggregate they do no more than make out a strong case; the suggestion that the streptococci are concomitants associated with some other unknown primary agent cannot be excluded with absolute certainty.
Can the analysis of the antigenic structure of the different haemolytic streptococci help the argument?

Stevens and Dochez in their latest article referring to the results of certain investigations state that "with the exception of Williams—who has obtained the lowest percentage of agglutinable strains among cultures from scarlatina, and who finds that numerous strains from streptococcal infections, not associated with scarlet fever, agglutinate with scarlatinal antisera, these authors agree that scarlatinal strains possess common agglutinative characteristics."

The demonstration that there is a distinct agglutinative group of haemolytic streptococci associated with scarlatina would furnish strong support to the presumption that scarlet fever is due to an infection with a haemolytic streptococcus. The discovery in other streptococcal infections of agglutinable strains would not in itself invalidate the hypothesis, since it could not be disputed that streptococci from scarlatina are able to set up secondary pyogenic infections.

In view of the results obtained independently by Smith and by me, the conception of a group of scarlatinal streptococci with common agglutinative characteristics is no longer tenable. We have shown that there are at least two types of streptococci of common occurrence in the throats of scarlet fever patients which give little or no cross-agglutination with their respective antisera.

Two consequences follow from this observation. In the first place it is impossible to maintain the thesis that the haemolytic streptococcus is the cause of scarlet fever on the ground that all streptococci from scarlatina will agglutinate with a serum prepared with one of them, and on the other hand specificity in relation to scarlet fever could not be denied to a strain because it failed to agglutinate with a particular scarlatinal antiserum.

A problem in some respects of a similar nature arose during the work on meningococci in the cerebro-spinal fever epidemic of 1915. Whilst there was little question that the meningococcus was the cause of cerebro-spinal fever, there was some doubt as to the relationship to the disease of certain Gram-negative cocci which were common in the nasopharynx of the general population. These cocci were culturally identical with meningococci, but they did not in general react with agglutinating sera prepared with the types obtained from military cases. Subsequently it was found that all cerebro-spinal fluid strains did not conform to the chief meningococcal types, and it was shown that these aberrant meningococci which occurred particularly in the meningitis of young children could be identified serologically with individual strains of the Gram-negative cocci from the nasopharynx of healthy carriers.

It is by work on similar lines that I have endeavoured to establish the significance of streptococci in scarlatina, so far as serological work alone can contribute to the solution of the problem. The aim of this investigation, as

previously pointed out, has been first to define the serological types among haemolytic streptococci from scarlatinal sources and then to determine their incidence in other conditions.

There have been shown to be three types of haemolytic streptococci which occur more frequently in the scarlatinal group than in the puerperal. One of these types, Type 2, has so far been found only in cases of scarlet fever or in contacts. These facts do suggest that the association of streptococci with scarlet fever is not accidental, but before deductions can safely be drawn from the distribution of the types it will be necessary to extend the range of inquiry to other forms of streptococcal infections.

In addition to the three types the scarlatinal group contains a number of strains with individual antigens, and a few of these strains give the same agglutination reactions as certain of the puerperal strains, though identity has not yet been established by absorption. The existence of this heterogeneous collection of strains in the scarlatinal group and their connection with the puerperal group raise the question of the relationship of haemolytic streptococci in general to scarlet fever.

It is perhaps premature to speculate on this subject before it has been finally proved, however much one may be inclined to favour the view, that scarlatina is a streptococcal infection. One may, however, interpret the results in the following way. Those strains of haemolytic streptococci which are found most frequently in scarlatinal throats and have been designated Types 1, 2 and 3 may represent the most infective strains or those specially suited by their antigenic structure to cause scarlet fever. The haemolytic streptococci from the heterogeneous group which is probably made up of a large number of different types, found also in puerperal septicemia and other pyogenic infections, may rarely cause scarlet fever and perhaps never cause large outbreaks. To revert to the analogy with meningococci mentioned earlier, the heterogeneous scarlatinal strains would stand in relation to scarlet fever in the same position as the nasopharyngeal meningococci amongst non-contacts do to cerebro-spinal fever. That is to say, their infective qualities are feeble and they give rise to scarlet fever only in specially favourable circumstances.

If these observations are well founded, it will be recognised that discrimination by serological methods between scarlatinal and non-scarlatinal streptococci is a difficult matter. The capacity of a strain to produce a toxin may be, as in the case of the diphtheria bacillus, an essential criterion.

**Summary.**

The analysis of a series of haemolytic streptococci of scarlatinal origin by agglutinin absorption experiments has revealed the existence of three chief types. Among 81 strains from scarlatinal throats 37 have been shown to belong to one or other of the three types; 12 have been classified as Type 1, 14 as Type 2 and 11 as Type 3. In addition, two strains have been found which absorbed the agglutinin from both Types 1 and 3 sera.
Types 1 and 3 are related types; Type 2 appears to be sharply defined and so far has been found only in association with scarlet fever.

Types 1 and 2 correspond with types of the same designation defined by Dr J. Smith in Aberdeen.

The puerperal group of haemolytic streptococci, of which 46 strains have been examined, comprises a number of antigenically individual strains, some of which are identical with each other. In addition, puerperal strains conforming to the chief scarlatinal types have been found, viz., 4 of Type 1 and 1 of Type 3.

In the scarlatinal group 42 strains failed to absorb the homologous agglutinin of any one of the three chief type sera. Agglutinating sera which have been prepared with seven of them agglutinate by the rapid method the homologous strain in each instance and fail to give a similar reaction with any of the three types. Thus, their dominant antigens are different from each other and from the chief type antigens, and the strains belong to a heterogeneous group.

The Dochez strain belongs to the heterogeneous group, and a single homologue, apart from 45/2 (Zingher), has been obtained from a case of cerebrospinal meningitis following scarlet fever.

Two strains from the heterogeneous scarlatinal group give typical rapid agglutination with serum from two of the puerperal strains.

Out of 9 strains from the throats of persons not known to have been in contact with scarlet fever, one belonged to Type 1. In comparison, from five throat swabs, taken at a school where there had been an outbreak of scarlet fever, two strains belonging to Type 2 and one to Type 3 have been obtained.

A rapid method of agglutination at room temperature with drops of a dilution of a powerful immune serum and a streptococcal suspension mixed together on a slide, gives a useful preliminary indication of type which should be confirmed by an absorption test.

The existence of a common agglutinative factor among scarlatinal strains has not been confirmed where the strains selected belong to the two chief types and form perfectly stable suspensions.

Puerperal fever has been shown in five instances to be caused by the chief type strains, 1 and 3, of the scarlatinal group.

In the light of the above data I have discussed the significance of streptococci in scarlatina and conclude that there is strong evidence of a causal relationship.

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