THE SEROLOGICAL CLASSIFICATION OF *STREPTOCOCCUS PYOGENES*

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Introduction

This study of haemolytic streptococci of human origin was begun by the serological investigation of a series of streptococcal cultures obtained from cases of scarlatina. The results, which were published in this *Journal* in 1926 and 1927, showed that scarlatinal streptococci were not uniform in serological character, though certain types defined by agglutination reactions predominated. The dominant types in a series of 222 strains were four in number and were designated Types 1, 2, 3 and 4; they occurred in the following proportions, viz. 4·5 per cent. (Type 1), 25·6 per cent. (Type 2), 20·3 per cent. (Type 3) and 19·8 per cent. (Type 4). The remaining 29·7 per cent. formed a heterogeneous group in which many serological types were represented; at

least nine types were defined, and certain of these, in addition to two of the chief types, were identified with streptococci of puerperal origin. The serological specificity of the four most prevalent types occurring in scarlet fever and their predominance in scarlatinal infection have been confirmed by Allison (1932), and Andrewes (1932) in a very careful study of the serological relationships of human haemolytic streptococci came to the conclusion that three, viz. Types 1, 2 and 4, were serological entities, but that the fourth, *i.e.* Type 3, was doubtful in regard to type-specificity. Smith (1926) obtained from 210 cases of scarlet fever in Scotland 119 Type 1 strains and 57 Type 2 strains; his results were published before mine, but a previous exchange of cultures had assured us that our two main types had received the same designations.

The serological analysis of the heterogeneous group of scarlatinal streptococci, *i.e.* the group made up of types which are less commonly found in scarlatina than the so-called chief types, has been continued in conjunction with investigations of outbreaks of streptococcal infections, both scarlatinal and non-scarlatinal, in schools and other institutions. The advantage of this plan of study has been that at the same time as the types were being established by laboratory methods the frequency of their occurrence, *i.e.* their epidemiological importance, as well as their association with various clinical conditions, was being estimated, and a type has not been considered as fully established until it has been encountered in several outbreaks of disease. From time to time, during the rather prolonged investigation which this plan has made necessary, references to the progress and the epidemiological value of the systematic classification have been made in the *Annual Reports of the Chief Medical Officer of the Ministry of Health* (1929 to 1933). The serological classification has helped to show, what has long been suspected on clinical and epidemiological grounds, that scarlet fever is only one of the manifestations of scarlatinal infection, infection now generally agreed to be streptococcal in nature, and that a variety of different streptococcal illnesses can be co-ordinated epidemiologically; these questions are discussed in a paper published in 1931 by Glover and Griffith on the sequels of tonsillitis. In 1928, Griffith and Gunn showed the frequency of re-infection with haemolytic streptococci among patients in a fever hospital, and suggested that such re-infection was the chief cause of second attacks and of late complications in scarlatina. They also showed correlation between the serological type of streptococcus and the severity of scarlatinal attack. In 1930, Glover and Griffith gave an account of a bacteriological investigation of an outbreak of scarlet fever due to Type 2 in a preparatory school. In the Chief Medical Officer's *Annual Reports*, 1931 and 1932, significant data are given as to the pathogenic activities of haemolytic streptococci in residential schools, and the possibility of an association between rheumatic fever and acute throat infections with haemolytic streptococci was suggested. In a discussion on the epidemiology of scarlet fever reference was made to a possible analogy from the epidemiological standpoint, particularly with regard to their sporadic incidence, between scarlet fever and

epidemic cerebro-spinal fever, and it was pointed out that the control of scarlet fever is only part of a much larger problem, that is, the control of infections with haemolytic streptococci in general. A trial of active antitoxic immunisation in the control of outbreaks of tonsillitis was suggested. Some examples were given of outbreaks of infection with different serological types of streptococci in semi-closed institutions, and the distribution of the types was again determined in 100 cases of scarlet fever, collected mainly in the first six months of 1932; out of the 100 strains obtained 25 belonged to Type 1, 10 to Type 2, 8 to Type 3 and 1 to Type 4; the remaining strains, hitherto placed in a heterogeneous group, were classified as follows: 1 to Type 5, 2 to Type 6, 16 to Type 8, 3 to Type 12, 2 to Type 13, 2 to Type 17, 2 to Type 27, and 28 unidentified. In these *Reports* it was pointed out that during the first half of 1932 the cases of scarlet fever were sporadic in distribution, and that this probably accounted for the unusually small proportion of the epidemic scarlatinal types. The relationship between streptococci from scarlatina and those from other forms of streptococcal infections was discussed in a note communicated to the permanent committee of the Office International d'Hygiène publique (1933).

In agreement with Andrewes, who has discussed the question of nomenclature of human haemolytic streptococci in the article already referred to, I have designated my streptococcal strains as types of *Streptococcus pyogenes*. It has seemed to me that the colonial appearances and the lytic activity on surface plates of horse blood agar are sufficiently characteristic to warrant their being placed in one group or species. There are undoubtedly certain strains of animal origin which are indistinguishable in the above respects from *Str. pyogenes*, but these, according to Lancefield (1933), who followed up the earlier observations of Hitchcock (1924), can be differentiated by means of a precipitin test from streptococci of human origin. The presumption is that they rarely infect man and perhaps never produce human disease. I have, however, borne in mind the possibility of encountering these animal strains as temporary inhabitants of the human throat, and it is on this account that I have considered as established pyogenes types only those strains of haemolytic streptococci which have been associated with outbreaks of disease in man.

The above statement with regard to the probable pathogenicity of these animal strains must be qualified in view of an important observation by Dr R. Hare, Queen Charlotte's Hospital Research Laboratories (personal communication). He has shown by precipitation tests that the strain "Azgazardah," which I have classified as Type 7 of the streptococcus pyogenes group, falls into Lancefield's Group C, a group hitherto made up entirely of haemolytic streptococci of animal origin. Strains of Type 7 are certainly pathogenic for man and have been obtained from cases of erysipelas and sore throat though not from scarlet fever (*vide infra* under Type 7). Harrison, "Niel" and "Angel," classed provisionally as Type 16, Type 20 and Type 21, may fall into the same category as Type 7.

By far the most satisfactory way of identifying an organism is to establish its serological type, and my results show in the first place that it is possible to make a permanent systematic classification of the *Str. pyogenes* group by

agglutination methods, and in the second that the numbers of epidemiologically significant types do not exceed a workable limit. In fact, the existence of a multiplicity of serological types of so widely distributed an organism as the *Str. pyogenes* has its advantages, in that it renders possible the co-ordination of different illnesses epidemiologically and the tracing of the paths of infection. I have already indicated some of the results which have been achieved in several fields of practical work with the aid afforded by the precise method of serological identification.

I. CULTURAL IDENTIFICATION

For the preliminary identification of *Str. pyogenes* the characters of the colonies on the surface of blood agar plates are helpful. For the blood agar plates I have continued to use horse blood and the plates have been prepared in the way described in my previous report: to nutrient agar, made with trypsinised meat broth, from 3 to 5 per cent. of fresh oxalated horse blood is added. The mixture is poured into plates in which a thin layer of agar without blood has been allowed to set, as recommended by M. H. Gordon. Occasionally strains of *Str. pyogenes* are met with which produce very slight areas of lysis around the colonies on a surface plate incubated aerobically. A plate therefore must be examined with care to avoid missing such slightly lytic colonies, and in particular the surface appearance of suspicious colonies should be noted. But these rare occurrences do not seem to me to make it necessary to use anaerobic methods as a routine procedure, since there is an added risk of confusing other species of haemolytic streptococci with *Str. pyogenes*. I have little to add to the description of the colonial appearances given in my earlier reports mentioned above. But I should remark (1) that colonies of the rough coherent first variety, instead of being disc-shaped, may be markedly irregular in outline; a few types, in particular Type 8, regularly grow in this shape, which however is not confined to particular serological types; (2) that there are other serological types in addition to Type 3 which have so far been found to produce only the second colonial variety (viz. soft, shiny colonies), or the third variety (viz. watery or mucinous). It should be noted that the third variety will only attain its full development as a clear dome-shaped colony, when the individual colonies are well spaced and the medium is moist; otherwise they are often thin, flattened and wrinkled, in which state they may be confused with the first variety. The essential distinction between the three varieties of colonies is in the grade of consistency of the bacterial growth; there is no sharp differentiation between them, and all three may be occasionally found on the same plate from a single strain. Each variety may be obtained in profuse culture on the first plate from a human inflammatory lesion, and, as far as man is concerned at least, no one variety denotes a lower degree of pathogenicity than another. What the actual cause of these variations in consistency may be is a mystery, but, as I will show later, there is some evidence that the softer the colony the greater is the tendency to loss of

serological type-specificity. This appears to be in agreement with the results of Todd and Lancefield (1928), although Todd lays stress on the surface appearance rather than on the consistency of the colonies; his matt colony clearly corresponds to the first variety, and his glossy colony to the second variety. Todd has noted that a matt culture may give rise to isolated large glossy forms, which he has termed pseudo-glossy; such colonies no doubt correspond to the third variety, which is probably the same as Cowan's smooth colony and resembles Davis's *Streptococcus epidemicus*. It should be remembered, however, that certain animal strains of streptococci produce clear watery colonies which are indistinguishable from similar streptococcus pyogenes colonies, though they can be differentiated by fermentation and, as shown by Lancefield (1933), by specific precipitin tests.

II. Serological identification

(*a*) *Preparation of agglutinating sera in rabbits*

In the procedure ordinarily used for the serological analysis of a bacterial group a number of sera are prepared from typical but arbitrarily selected strains. Subsequently the choice of strains is made from those which cannot be identified serologically by tests with the first sera. In order to obtain typical strains, as the colonial characters of the primary cultures of streptococci often give no definite indication of the antigenic qualities of the strain, I have found it best to select the streptococci from acute cases of disease in preference to carrier strains. As a precaution against loss of type specificity it is advisable to use an early subculture and to prepare from it the whole amount of vaccine required for treatment of the rabbit. In the case of three types, Nos. 3, 13 and S.F. 73/4 (19), which tend to partial degradation in culture, such vaccines have been made from selected type-specific colonies, and undoubtedly this would be preferable for all types which are serologically unstable.

In the preparation of immune sera in rabbits it is recognised that the capacity of the individual animal to respond to treatment is the chief factor, though dosage, and in particular the spacing of doses, appears to influence the result. The effect of the injections on the general condition of the animals must be watched, since prolonged treatment is often necessary; probably when response is slow it would be more economical in time and effort to reject the animal and start afresh.

The following treatment has resulted in the production of satisfactory antisera, though it must be confessed that the failures have more than equalled the successes. The suspensions for injection have been prepared from stock cultures maintained in the refrigerator after a single night's incubation in blood broth, and subcultured when required in nutrient broth. The broth cultures, after being heated to 60° C. for one hour, were centrifuged and the deposits re-suspended in salt solution. Three injections on successive days were given intravenously, followed by a rest of four days, this procedure being

continued for six weeks, when a test bleeding was made. If the type-specific titre was not satisfactory the injections were repeated for a further six weeks or longer. The doses were gradually increased until the animal was receiving on each of the three days the deposit of 15 c.c., or even 30 c.c., of broth culture. The animal was bled when a preliminary test showed that it had a sufficiently high titre of type-specific antibodies. The standard aimed at was such that a tiny loopful of a 1 in 5 dilution of the serum which had been absorbed with a mixture of known heterologous strains to remove group agglutinins (*vide infra*) should give rapid flocculation with a drop of the homologous suspension on a slide. The titration of a crude serum against the homologous suspension does not necessarily give an accurate indication of the content of the serum in type-specific antibodies; it may do so, if the suspension in use is wholly type-specific, but haemolytic streptococci are often very sensitive to group agglutinins, so that these must first be removed by a preliminary absorption.

(*b*) *Suspensions for agglutination tests*

The preparation of suspensions suitable for agglutination has always been the chief difficulty in the typing of streptococci. The advantage of the slide method of agglutination which I employ is that it does not require suspensions so uniform and stable as the water-bath method, and there are comparatively few strains, at first too granular for test, which have remained completely refractory. In the testing of an unknown strain it has been the custom to take colonies from the primary plate cultures and to make subcultures in each of the following three fluid media, viz. (1) plain trypsinised meat broth, (2) the same broth with 5 per cent. serum (bovine or horse), and (3) the same broth with 5 per cent. ascitic fluid. Plain broth cultures are more sensitive to the action of agglutinating sera and more liable to grow in a granular fashion than ascites broth cultures. The growth of *Str. pyogenes* in ascites broth is generally uniform or readily re-suspended, but unless the strain is strongly type-specific, or its appropriate agglutinating serum is of a high titre, no agglutination is obtained; a negative result with ascites broth culture has therefore no diagnostic value. Serum broth cultures, which occupy an intermediate position in the above respects between plain and ascites broth, are useful for certain types. If the plain broth culture is too granular to make a suitable emulsion, the growths in the other two media are tried. In the event of the cultures in the latter two media either being unsuitable or giving negative agglutination reactions, successive subcultures are made in the plain broth medium until the strain forms a uniform suspension. It is sometimes useful to plate an obstinately granular culture and to subculture a number of colonies, selecting for preference those which show differences in appearance; for instance, the soft, shiny or watery colonies may give better suspensions than the coherent colonies. I have avoided any treatment of the suspensions themselves, as one cannot be certain at present how the type-specificity might

be affected, and have confined my efforts to obtaining a suitable medium by alterations in its constituents and its reactions. It appears, however, to be inherent in some types to grow uniformly, and in others to grow in a granular fashion, and frequent passage through fluid media is at present the only solution of the latter difficulty. One recognises that such passage may result in some loss of type-specificity and increased sensitiveness to group agglutinins, but this is relatively unimportant when the test agglutinating sera are powerful and highly type-specific.

(*c*) *Agglutination technique*

The slide method of agglutination described in previous reports was at first adopted tentatively and simply as a means of avoiding certain difficulties, but experience has shown that by its use serological types of streptococci can be defined with certainty, and that strains can be identified more rapidly than in any other way. If the agglutinating sera are of a high titre, and both sera and strains are highly type-specific, there is immediate coarse flocculation when the suspension comes in contact with the appropriate serum; the absence of any change on the application of heterologous sera is a striking contrast. Not all strains, however, are equally type-specific even when freshly isolated, and, moreover, agglutinating sera invariably contain some agglutinins which act upon heterologous members of the pyogenes group. It is necessary, therefore, as already mentioned, to remove or greatly to reduce the group agglutinins in a serum by treatment with heterologous antigens. In preparing sera for routine diagnostic use I have treated each type serum in a dilution of 1 in 2·5 or 1 in 5, according to its strength, with mixtures of the majority of the other type strains. After the first treatment all the type strains were tested against all the sera to ascertain if the heterologous group agglutinins had been removed, and when necessary particular sera were treated a second time. Even when the tests against the stock type strains indicated an apparently complete removal of heterologous agglutinins, it might still be necessary to be on guard against group reactions in the case of specially sensitive new strains. Such difficulties are less likely to arise if care is taken to put into use only sera with a high type-specific titre.

(*d*) *Confirmation by agglutinin-absorption experiments*

One may be left in doubt as to the type of a strain when it gives some agglutination with more than one of the purified type sera. Theoretically, a simple agglutination test with a single dilution of a pure type-specific serum should be sufficient to identify the type of a strain, but there appears to be a variety of group antigenic components, and all the corresponding antibodies may not be removed by the treatment with the selected heterologous strains. In such instances a confirmatory test of type must be made by the usual method of absorption of homologous agglutinin; the test of its removal may be made by the slide method or in the water bath.

An unexpected complication, which has not yet been explained, was discovered in the course of such absorption experiments. It was found that a few, viz. Types 7, 16, "Angel" (21) and "Niel" (20), out of the total number of types, had the power of greatly diminishing or completely removing the agglutinating property of heterologous sera for their homologous strains. Yet these strains are undoubtedly individual types; there is no cross-agglutination between them, even when crude sera are used, and they are not agglutinated by those sera from which they are able to remove the agglutinin by one or more treatments. Strains such as these must obviously be omitted when purifying type sera by absorption.

(*e*) *Abbreviation of procedure for identification of type*

The testing of an unknown strain with at least twenty-seven different sera is a laborious and time-consuming task, especially when one is dealing with an outbreak involving the examination of a large number of strains. To avoid coming to a premature conclusion on a positive agglutination test, it is advisable as a general rule that all the test sera should be tested against every unknown strain. With a battery of thirty sera, for example, this may be accomplished quite speedily by mixing the purified sera in groups of five, so that every strain would be tested against six grouped sera. When a positive reaction is obtained with one of the grouped sera each of the five individual sera contained in it are put up against the strain under examination.

III. SEROLOGICAL TYPES OF *STR. PYOGENES*

The strains of streptococci which have served as the material for this study have been derived from cases of scarlet fever and tonsillitis, and to a less extent from otitis media, puerperal fever and a few other streptococcal conditions. The primary object in view was to define the serological types which make up the *Str. pyogenes* group, and incidentally to determine how far these types could be correlated with different clinical conditions. The human throat is apparently the natural habitat of the *Str. pyogenes*. It is the seat of the primary infection in such diseases as scarlet fever (excepting scarlet fever of surgical and puerperal origin and that following burns), tonsillitis and otitis media, and it is the focus from which the great majority of streptococcal infections are directly spread. In fact, all the types so far defined and encountered in the many outbreaks of disease which have been investigated have been obtained from throat swabs, and with a few exceptions from cases of scarlet fever. Agglutinating sera from twenty-seven strains of haemolytic streptococci with individualistic serological characteristics are being employed at the present stage for the identification of unknown streptococcal strains. Not all these strains can be said to be equally well established as definite *pyogenes* types, since a few have not complied with an essential criterion already mentioned, viz. that their epidemiological importance should have

been demonstrated, and there is a possibility that they may belong to a different group. Moreover, there still remains a residue of strains which cannot be identified by means of the above sera. Whether it is that these unidentified strains are independent types, distinct from the above, or partly degraded representatives of those types, is still under enquiry. Excepting this small but not negligible residue, it is possible with the aid of the above twenty-seven sera to determine the serological type of any freshly isolated strain of *Str. pyogenes*. The streptococci from epidemics and small outbreaks, scarlatinal and non-scarlatinal, are most readily identified, whilst the strains which give trouble are commonly derived from sporadic cases of disease and from carriers.

The following is a list of the types so far defined, some provisionally, under each of which is given a short account of the epidemiological features that have been observed, together with any colonial or other peculiarities which might be an aid to identification. It is recognised, however, that the minor peculiarities which have distinguished type strains obtained mainly around London may not be shown by the same types from other parts of the country and from abroad. Records of the occurrence of types abroad depend upon their identification in cultures sent to me by the various workers named, with a few exceptions which are stated.

IV. List of serological types of *Str. pyogenes*

Type 1

One of the chief types associated with epidemic scarlet fever, occurring in about 10 per cent. of scarlatinal cases in this country. It has been identified in U.S.A. (Anna Williams, 1932; Mueller and Klise, 1932). It may be non-toxigenic or weakly toxigenic, since it has been found in outbreaks of tonsillitis without rash.

In primary cultures colonies of all three varieties, *i.e.* opaque and coherent, soft and translucent, and watery or mucinous, have been observed. Early subcultures in broth are frequently too granular and unstable for agglutination. Serum broth is a useful medium, and ascites broth gives perfect suspensions, which however do not agglutinate strongly with the type-specific serum.

Occurrence in outbreaks, i.e. series of cases epidemiologically connected, and sporadically

Schools. N.B. Outbreak of 13 cases, viz. 10 scarlet fever, 1 otitis media, 1 influenza, 1 sore throat.
U.B. 1 tonsillitis, 1 otitis media.
M. 3 cases of scarlet fever.
B. 9 cases; scarlet fever or sore throat.
C/B. 4 cases of rhinitis and 4 throat infections; no rashes.
J.B. 16 cases of scarlet fever.
Q.B. Extensive outbreak of throat infections, some severe clinical cases; a few atypical rashes.
D/B. 40 cases of tonsillitis and sore throats; rash in one mastoid case.
F.B. 3 sore throats.
Dr C. Ponder. Puerperal patient and nurse's throat.

Worthing. Scarlet fever outbreak; 6 cases.
Dr L. H. D. Thornton. Cultures from septicaemia in a milker and from the milk of cows; Golledge (1932).
Q.M. Hospital. 1 sore throat.
St G. Hospital. 5 cases of scarlet fever.
Family outbreak. Scarlet fever and sore throats.
Family outbreak. 1 sore throat without rash.
Foreign strains. U.S.A., S 3, Dochez, Avery and Lancefield (1919); Sc. Type 1, 2/2 (Anna Williams); New York, C 203; New Haven, 2 strains from Miss A. R. Beebe; Russia, 1 scarlet fever; Switzerland.

Type 2

One of the chief types associated with scarlet fever, occurring in about 20 per cent. of scarlatinal cases in this country. It has been isolated in U.S.A. by Mueller and Klise (1932), but not by Anna Williams (1932). All three varieties of colonies have been obtained on primary plates from human lesions. Suspensions almost invariably good in all media; strong agglutination reactions with homologous type-specific serum. It occurs occasionally in non-toxigenic form, *i.e.* it has caused severe outbreaks of throat infection without rashes.

Occurrence

Schools. T/B. 3 cases of scarlet fever.
T/B. 6 cases of scarlet fever.
M/B. 2 cases of scarlet fever.
T. 12 cases of scarlet fever.
J.B. 2 cases; post-nasal catarrh, tonsillitis.
C.G. 3 cases of scarlet fever.
Prep. School. 17 cases; tonsillitis, sore throats, naso-pharyngitis; no rashes.
Prep. School. Scarlatinal outbreak; Glover and Griffith (1930).
W. 3 cases of scarlet fever at a private school.
R.B. 45 cases; pyrexial sore throats; 1 doubtful rash.
St G. Hospital. 3 cases of scarlet fever.
Brookfield. 7 cases of scarlet fever and sore throats.
Stannington. 170 cases of scarlet fever; ? milk outbreak.
Scarlet fever in Huddersfield, Eastbourne, Farnborough.
Throat of a nurse whose puerperal patient died.
Suspected scarlet fever carrier at West Bromwich in 1930.
Scarlet fever outbreak at a dairy in Hove, obtained from 6 persons.
Tonsillitis case with epistaxis and haematuria.
Boy with blood and casts in urine; culture from urine.
Bucks. Case of otitis media.
Cultures from joint pus following scarlet fever.
Dr H. J. Gibson (1933). Acute rheumatism, No. 36.
Foreign strains. Dr A. F. Coburn's strains, R 14 and R 53 (1932).

Type 3

One of the chief types associated with scarlet fever, occurring in about 20 per cent. of scarlatinal cases in this country, while it appears to be one of the commonest types in U.S.A. (Williams, Beebe, Mueller and Klise). In primary cultures strains in this country generally form soft translucent or watery mucinous colonies (second and third varieties), but recently the opaque

coherent variety of colony has also been found, particularly in cultures from U.S.A. Suspensions are generally finely granular in trypsinised meat broth, very granular in serum broth, and good in ascites broth. Type 3 tends to lose its type-specific qualities and to throw off group colonies, but the type-specific colonies give in broth subculture good coarse flocculation with the type serum.

Occurrence

Schools. O/B. 8 cases in one term, viz. 5 with rashes, 1 desquamated, 1 tonsillitis, 1 sore throat.
B/B. 2 cases of scarlet fever in 1933.
U.B. 8 cases; 3 scarlet fever, 3 tonsillitis, 2 otitis media.
D/B. 1 case of scarlet fever, 1 sore throat.
R.B. 5 cases of scarlet fever, 2 sore throats.
L/B. 3 cases of follicular tonsillitis; no rashes.
St G. Hospital. 1 case of scarlet fever.
Brookfield. 3 cases of scarlet fever.
Oxford. 8 cases of scarlet fever.
Dr A. G. Signy. 2 cases of scarlet fever and 1 carrier.
Scunthorpe. Scarlet fever prevalence in 1929, obtained from 9 cases.
Whitehall cottages. Outbreak of scarlet fever; 6 carriers found, 2 of whom subsequently developed scarlet fever.
Restaurant outbreak of scarlet fever. Cultures of Type 3 obtained from 5 cases.
Type 3 obtained from lobar pneumonia complicating scarlet fever (no pneumococci in sputum).
Prof. H. B. Maitland. Case of erysipelas, Davis.
Foreign strains. S 84, Dochez, Avery and Lancefield; Dick 1 and 2 (cultures sent by Mrs Maitland); Sc. Type 1, 28/1, 55/4, 4/2, 47/2, A.W. 64, 35/2 (Anna Williams, 1932); New Haven, 12 strains from Miss A. R. Beebe.

Type 4

Variable frequency in scarlatina, up to 10 per cent. It almost invariably causes a mild disease with an evanescent rash. In primary culture the colonies may be watery or mucinous but are generally opaque, coherent and slightly irregular in outline; frequently the zones of haemolysis around the colonies on horse blood agar are very slight. Uniform suspensions for agglutination are difficult to obtain in plain broth; ascites broth suspensions are good, but with them the type serum rarely gives agglutination.

Occurrence

Separate outbreaks have been uncommon.
St G. Hospital. 31 cases; mainly scarlet fever.
2 cases of tonsillitis.
Dr H. J. Gibson. Case of acute rheumatism.
Severe sore throat in a nurse in a scarlatinal ward.
School R/B. 1 case of otitis.
Taunton. 6 cases of sore throat, 1 case of otitis media.
Foreign strains. Type 4 strains have been obtained from U.S.A., viz. Park 27; A.W. Erys. Type 2, Sub a 314 and A.W. Sc. Type 4, 28/1 (Anna Williams); R 25, Group 1, Dr Coburn; and cultures Nos. 22 and 70 from Dr A. Grumbach, Switzerland.

Type 5

The original type strain ("Franklin") was obtained from a case of puerperal fever by Dr L. Colebrook. It is a common tonsillitis strain, and is occasionally found in sporadic cases of scarlet fever; frequent cause of otitis media. Suspensions for agglutination are generally good in all three media (broth, serum broth and ascites broth), and the type serum gives strong agglutination reactions.

Occurrence

Schools. O/B. 1 sore throat, 1 otitis media.
F/B. 2 cases of otitis media.
R/B. 6 cases; tonsillitis 4, carriers 2.
Q/B. 3 sore throats (2 developed otitis media).
J.B. 1 tonsillitis.
C.G. 9 cases in Lent (tonsillitis 5, otitis media 3, scarlet fever 1); 8 more cases in Summer; no rashes.
1 Prep. School. Outbreak after influenza, 3 scarlatinal rashes, 6 broncho-pneumonia, 10 otitis media, 7 mastoid.
2 Prep. School. Outbreak of throat infection, 1 otitis media.
Dr J. Smith. Puerperal fever, No. 23.
Peterborough. 4 cases of scarlet fever, 1 sore throat.
Worthing. 3 cases of sore throat.
Q.M. Hospital. 5 sore throats in rheumatic children.
St G. Hospital. Cultures from 3 throats.
Brookfield. 5 sore throats (1 considered scarlet fever).
P.M. Endocarditis.
Dr G. H. Eagles. Aronson streptococcus.
Foreign strains. Dr A. F. Coburn's U.S.A. cultures, viz. R 3, R 28, R 51, R 56.

Type 6

Original type strain ("283 T") was obtained from the throat of a scarlet fever case. It is, like Type 5, a common strain in tonsillitis and otitis media, and is occasionally found in sporadic scarlet fever. Suspensions in plain broth are often partly uniform, partly granular, but good in serum and ascites broth. Cultures in all three media give coarse masses with the type agglutinating serum.

Occurrence

Schools. F/B. 17 cases; tonsillitis 10, otitis 4, pneumonia 1, impetigo 2.
A.G. 1 otitis media.
Q.B. 14 cases of tonsillitis and pharyngitis in two consecutive terms.
D.B. 18 cases of tonsillitis (3 followed by otitis media; 1 rash).
L.B. 2 cases of purulent nasal discharge.
G.G. 6 cases of otitis media in one term.
U.B. 1 scarlet fever, 1 tonsillitis, 1 otitis media in Lent, 1934.
R.B. 1 case of meningitis.
H.G. 2 sore throats.
A.G. 1 case of otitis media.
W.D. 7 cases of sore throat and tonsillitis.
Dr J. Smith, Aberdeen. 2 cases of puerperal fever, Nos. 37 and 40.
Dr H. J. Gibson. 2 cases of acute rheumatism, Nos. 56 and 88.
Maternity Home. Throats of 2 nurses.
Suspected scarlet fever carrier. Cultures from nose and throat.

Severe sore throat in a nurse in a scarlet fever ward.
G. School. 1 case of scarlet fever.
Foreign strains. S 43, Dochez, Avery and Lancefield. (Dr Todd's matt, glossy and matt virulent colonies all gave type-specific reactions.) Dr A. F. Coburn's rheumatic strains, R 8 and R 27.

Type 7

The original strain ("Azgazardah") was obtained from puerperal fever by Dr L. Colebrook. This type has not been found in scarlet fever. It is not uncommon in sore throats, and has been found in small numbers in normal throats; it appears to be common in erysipelas in U.S.A. It, "Niel" and some "Angel" strains are the only *Str. pyogenes* types in my experience which are highly sensitive to the bacteriophage of Clark and Clark, modified by Shwartzman. Good suspensions are commonly formed in all three media. The original type strain is very type-specific and produces a type-specific serum; dense clumps are formed when serum and suspension come into contact.

Occurrence

This type is usually sporadic, but at
School R.B. it was found in profuse culture in 3 cases of sore throat in one term.
School D.B. 3 throat carriers (it was doubtful if there was any local disease).
School Q.B. 1 sore throat.
W.D. Prep. School. 1 sparse carrier.
1 normal throat carrier.
Hertford. 1 carrier.
Peterborough. 1 carrier (a nurse).
London. 1 carrier (a child).
Foreign strains. No. 2616 Birkhaug, National Type Collection; A.W. Erys. Type 2 B 3 (Anna Williams); E 1, E 2 (Shwartzman).

Type 8

This is a type ("S.F. 4") which is commonly associated with scarlet fever. It first came to notice in the course of an investigation of 100 cases of scarlet fever in 1932, when it was obtained from sixteen cases. The primary colonies are almost invariably opaque, coherent and markedly irregular in outline; zones of lysis on horse blood agar may be very slight. Suspensions in plain trypsinised meat broth are too granular for agglutination in early subcultures; ascites broth suspensions are uniform and give a good type-specific reaction.

Occurrence

School U.B. 1 case of scarlet fever.
O.B. 1 case of scarlet fever.
T.B. 1 throat carrier.
Peterborough. 3 cases of scarlet fever.
General Hospital. 3 cases of scarlet fever (2 cases after tonsillectomy and 1 after contact with above).
Case of haematuria (following scarlet fever) of 2 months' duration; culture from urine.
Q.M. Hospital. 2 cases of sore throat.
Dr H. J. Gibson. Acute rheumatism, No. 27 A.
Foreign strain. Dr A. F. Coburn, S 6, Group 1.

Type 9

This type ("Symons") was first obtained from an extensive outbreak of sore throats in a public school, and was identified with the strain from the large milk outbreak of throat infections in Sussex, 1930. Primary colonies may be opaque and irregular, or watery or mucinous. Suspensions inclined to be granular.

Occurrence

School R.B. 26 cases; tonsillitis without rash.
Brighton and Hove. Outbreak of milk-borne streptococcal infections in over 1000 families with 65 deaths.
School J. B. 1 case of tonsillitis.
Q.M. Hospital. 2 cases of sore throat in rheumatic children.
Hampstead. 2 cases of acute rheumatism.
1 case of meningitis.
A case of persistent haematuria.
Foreign strain. Dr A. F. Coburn, R 55.

Type 10

The original type strain ("Dochez") was obtained from Dr R. A. O'Brien. It is an uncommon type in this country, and has not been found in scarlet fever. It is apparently more common in U.S.A. The colonies of the type strain are clear and mucinous; suspensions are granular.

Occurrence

There was one outbreak of sore throats in School Q.B. in 1927, and Type 10 was obtained from 12 cases.
Foreign strains. 3 strains from scarlet fever in Russia; Dr A. Wadsworth, E 14; A.W. Sc. Type 3, 57/2, A.W. 56, A.W. 17 B (Anna Williams); N.Y. 5, U.S.A., received from Sir F. W. Andrewes in 1931.

Type 11

This type ("Blackmore") was originally obtained from an outbreak of scarlet fever, and the strains collected so far have been mainly toxigenic. It is generally agglutinated by Type 12 serum, and may be confused with Type 12, though each appears to have a separate and individual type-specific antigen. Suspensions are generally finely granular, but suitable for agglutination. Primary colonies are often opaque, coherent and irregular in outline.

Occurrence

Schools. T.B. Outbreak of scarlet fever, 6 cases; persistent carriers.
Q.B. 3 cases; 1 tonsillitis, 2 scarlet fever (one developing otitis media).
G.B. 1 case of scarlet fever.
H.B. 1 case of tonsillitis.
Prep. School. W.D. 1 case of sore throat.
R.B. Lent, 1931, 5 cases; 3 scarlet fever, 2 tonsillitis.
R.B. Lent, 1933, 4 cases of scarlet fever.
Foreign strain. Dr A. F. Coburn, S 7, Group 1.

Type 12

This type ("S.F. 42") was first obtained from a case of scarlet fever. Primary colonies are generally opaque and coherent. In broth, suspensions from early subcultures are granular: ascites broth suspensions are generally uniform and frequently give a good type-specific agglutination reaction.

Occurrence

Schools. O.B. 1 case of follicular tonsillitis.
J.B. Pyrexia, pure throat culture.
R.B. Lent, 1934, 6 cases of tonsillitis.
R.B. Summer, 1934, 9 cases; 6 tonsillitis, 3 profuse cultures in rubella.
F.B. 2 cases of tonsillitis.
C.B. Lent, 1932, 4 cases of otitis media.
C.B. Summer, 1932, 1 case of otitis media.
O.B. Lent, 1934, 6 cases of sore throat and tonsillitis; no rashes.
O.B. Summer, 1934, 1 case of follicular tonsillitis.
Case of purulent peritonitis; surgical theatre sister was a carrier.
Essex. Scarlatinal outbreak; 6 cases in children.
Peterborough. 3 cases of scarlet fever.
Dr J. Smith's cultures, Nos. 30, 43 and 45, from puerperal fever.
1 case of otitis media.
3 throat carriers.
Dr H. J. Gibson's cultures from acute rheumatism, Nos. 67 and 67 A.
Single case of otitis media.

Type 13

This type was first obtained in a severe outbreak of tonsillitis at a home for rheumatic children and was designated "Carter" type. It has been the causal organism in a considerable number of streptococcal outbreaks. It has been found in occasional sporadic cases of scarlet fever.

In primary cultures the colonies have been invariably of the watery or mucinous variety (third variety). It has a tendency to lose its type-specific quality, and non-reacting colonies have been found in primary cultures from the throat. Suspensions in trypsinised meat broth are fair and generally suitable for agglutination. The type serum does not agglutinate ascites broth suspensions.

Occurrence

Schools. F.G. 4 cases; 1 sore throat, 2 follicular tonsillitis, 1 otitis media.
G.G. 9 cases of throat infection (1 developed otitis media).
D.B. Outbreak of tonsillitis and sore throats; 38 cases in two consecutive terms.
Q.B. Outbreak of tonsillitis, coryza and post-nasal catarrh; 21 cases.
Prep. School. Outbreak of tonsillitis with high pyrexia; 13 cases (3 developed otitis media, 2 broncho-pneumonia).
Taunton. 11 cases of sore throat.
Sussex. In a school of 90 children, 15 cases, including tonsillitis, scarlet fever (2), otitis media (4).
Q.M. Hospital. Obtained in 13 instances from throats over a period of 9 months.
T.B. 7 cases of sore throat; 3 cases of otitis media.
Cleethorpes. Outbreak of puerperal fever, 6 cases.
Dr J. Smith's cultures from puerperal fever, 1 instance, No. 59.

Outbreak of throat infections at a home for rheumatic children.
Two different family outbreaks.
Dr H. J. Gibson's cultures from acute rheumatism, 1 instance, Carter.
Brookfield. 1 sore throat.
Worthing. 1 carrier.
Foreign strains. Dr A. F. Coburn, R 24 and Terentino.

Type 14

The type strain ("Barker") was a culture from puerperal fever and was obtained by Dr L. Colebrook. Primary colonies are often of the third variety.

Occurrence

Schools. F.B. 25 cases of tonsillitis, pharyngitis, etc., in two consecutive terms, followed by otitis media in 3 instances.
U.B. 1 case of otitis media.
Q.M. Hospital. 3 cases of tonsillitis: 1 fatal carditis.
Single case of puerperal fever.
Single case of puerperal fever.
Dr H. J. Gibson's acute rheumatism cultures, 3 instances, viz. 13*dx*, 71 and 45*a*.
Foreign strains. Culture from scarlet fever at Nish, Jugoslavia; S. 23, Dochez, Avery and Lancefield (presented by Dr Todd); A.W. Erys. Type 2, sub 6, 420 (Anna Williams).

Type 15

Originally obtained from a case of scarlet fever at Liverpool and designated "J.S. 5." Primary colonies are opaque and coherent, and occasionally mucinous. Suspensions in plain broth are granular and difficult to work with.

Occurrence

School Q.B. 14 cases of tonsillitis (otitis media 1).
School J.B. 1 case of sore throat.
Puerperal fever. Dr G. H. Eagles's culture (No. 2368 National Type Collection).
Foreign strains. Dr A. F. Coburn's cultures, R 11, M 1, Nurse Hunt.

Type 16

This type ("Harrison") was obtained from a case of puerperal fever and from the throat of the nurse in attendance. Primary colonies are opaque and slightly irregular. Poor suspensions in broth and pronounced coarse flocculation with the type serum.

Occurrence

Schools. F.B. 1 case of tonsillitis.
R.B. 1 case of tonsillitis.
Q.B. 1 throat infection, pharyngitis.
Puerperal fever case and carrier nurse in 1930.

Type 17

This type ("Beatty") was first obtained from an outbreak of tonsillitis and otitis media followed by acute rheumatism at a public school. It is a difficult type to identify with certainty, as cultures in fluid media are very granular;

there is also group agglutination between Types 15, 17 and 18, but from absorption experiments each seems to be an independent type.

Occurrence

School D/B. 12 cases (5 tonsillitis, 7 otitis media; complications: meningitis and acute rheumatism).
Q.M. Hospital. From sore throats in 3 rheumatic children.
2 cases of scarlet fever in a series of 100 cases.
School J.B. 1 case of otitis media.
Dr H. J. Gibson's culture from acute rheumatism, No. 62.
Foreign strain. Dr A. F. Coburn's culture from acute rheumatism, R 9.

Type 18

This type ("Hutchinson") was first obtained from an outbreak of throat infections at a public school. Cultures show tendency to lose type-specificity and give confusing cross-agglutination with Types 15 and 17.

Occurrence

School D/B. 63 cases of pyrexial sore throats; no rashes, no otitis media.
School F.B. 54 cases of tonsillitis in two consecutive terms; complications: otitis media 1, subacute rheumatism 4.
Puerperal fever cultures (patient and nurse), National Type Collection, Nos. 2666 and 2667.
Foreign strains. Dr A. F. Coburn's cultures from acute rheumatism, R 18 and R 20.

"S.F. 73/4" (Provisional Type 19)

The strain "S.F. 73/4" was originally obtained from a case of scarlet fever. It forms watery or mucinous colonies, which are differentiated into group and type-specific varieties. The type-specific variety appears to be different from any other type, but it has been identified in only a few isolated instances and cannot yet be considered as an established type. *Occurrence Eagles, Erysipelas No.* 2365, *National Type Collection.*

"Niel" (Provisional Type 20)

This strain was obtained by Dr L. Colebrook from the throat of a house surgeon without definite signs of disease; it was present in the throat in considerable numbers. The strain "Niel" seems to be an independent type and gives good firm clumps with the type serum. This strain is phage-sensitive like Type 7.

"Angel" (Provisional Type 21)

This strain was obtained from a case of scarlet fever in the course of an outbreak at a home for children in Essex. The predominating type in that outbreak was Type 12, but in addition there were several strains, of which "Angel" was one, which could not be identified serologically. An agglutinating serum was made from "Angel," which appears to be a type-specific strain. Some strains of this type have been found to be phage-sensitive.

Type 22

This type ("63 T.") was first obtained from a case of scarlet fever. The primary colonies are opaque and generally slightly irregular in outline.

Occurrence

Schools. O.B. 1 case of nasal catarrh.
F.B. 6 cases of simple and follicular tonsillitis.
Q.B. 2 cases of sore throat; 1 case of tonsillitis.
J.B. 1 case of tonsillitis.
Q.M. Hospital. 2 sore throats in convalescent rheumatic children.
Brookfield. 2 sore throats.
1 case of endocarditis.
Seaford. 1 septic throat.
Family outbreak. 3 cases; mild tonsillitis, ear discharge, acute tonsillitis.

"King" (Provisional Type 23)

The "King" type was first obtained from an outbreak of scarlet fever and tonsillitis (4 cases in all) at a girls' school. Also from 3 cases of scarlet fever and 1 case of tonsillitis at a boys' school.

"Sylvia Turton" (Provisional Type 24)

This strain appears to be a separate and individual serological type.

Occurrence

Q.M. Hospital. 3 cases of sore throat in convalescent rheumatic children.
Foreign strain. Culture from Dr A. F. Coburn, R 23, Group 1.

Type 25

This type ("Matthews") was first obtained from a normal throat, the swab yielding a few irregular colonies.

Occurrence

Schools. R.B. 4 throat infections in Lent, 1934, and 1 case of tonsillitis in Summer, 1934.
C.G. 1 case of sore throat.
U.B. 2 cases of tonsillitis.
H.B. 1 case of tonsillitis.
Foreign strain. A.W. puerperal fever, Tl. 201 (Anna Williams).

"S.F. 13" (Provisional Type 26)

"S.F. 13" was first obtained from a case of scarlet fever, in a series of 100 cases examined in 1932.

Occurrence

1 case of tonsillitis (sparse colonies).
Foreign strains. Dr A. F. Coburn's cultures from acute rheumatism, R 12 and N 2.

Type 27

The type strain ("S.F. 40") was first obtained from a case of scarlet fever in a series of 100 cases examined in 1932.

Occurrence

Schools. U.B. 1 case of scarlet fever.

D.B. 1 case of tonsillitis; 2 throat carriers (sparse colonies).

From throats of 2 nurses associated with an outbreak of puerperal sepsis (no cultures from puerperal cases for comparison).

V. Type-specific and group characters of *Str. pyogenes*

The occurrence of bacteria in two phases, which he termed respectively group and specific, was first demonstrated by Andrewes (1922) in an ordinary culture of certain types of Salmonella. There are immunological variations, which I believe to have similar bearings on the question of serological types, in the *Str. pyogenes* group, although in streptococci there is no question of flagellar antigen, and I propose to make use of the terms type-specific and group in my description of these differential features. This variability in regard to type-specificity does not appear to take place, or at any rate cannot readily be demonstrated, in all the serological types of streptococci, and the most clear cut results have been given by Type 3. The agglutination reactions of Type 3 have frequently been rather difficult to interpret, and for some time I was uncertain whether the type had been correctly defined, that is to say, whether the agglutinin remaining in a Type 3 serum after treatment with heterologous strains was in fact type-specific. Andrewes (1932) indeed came to the conclusion after a prolonged study that while the individuality of each of Types 1, 2 and 4 was readily confirmed, Type 3 did not seem entitled to rank as a distinct entity. Prior to the observations which I am about to describe, my belief in the existence of a type-specific antigen in those strains identified as Type 3 was sustained by the fact that the agglutination reactions with freshly isolated strains from acute cases of scarlet fever were strongly marked with Type 3 serum and negative with presumably heterologous sera. It seemed clear that the anomalous results of agglutination and absorption experiments with the stock cultures of Type 3 must be due to changes occurring in the strains after subcultivation, and not to lack of type-specific agglutinins in the sera used.

(*a*) *Agglutination experiments with colony cultures of Type* 3

Numerous experiments were made with stock strains of Type 3 which gave little or no agglutination with a Type 3 serum rendered almost purely type-specific by treatment (absorption of agglutinin) with a mixture of heterologous strains. The Type 3 cultures were plated on horse blood agar, and a number of colonies were grown in broth and tested by the drop method of agglutination

on the slide with a type-specific Type 3 serum so prepared. Some colony cultures gave immediate coarse flocculation on the addition of serum, some a trace of agglutination, and others were completely negative. The strongly reacting cultures were evidently type-specific, since one, when tested by the slide method against thirty-four crude agglutinating sera, agglutinated only with the homologous serum. The proportion of type-specific colonies obtained from the stock Type 3 cultures on different occasions varied, and in one instance only one out of twelve reacted specifically.

(*b*) *Agglutinin-absorption experiments with colony cultures of Type* 3

A stable suspension for the test of absorption was prepared in glucose phosphate broth from each of two colony cultures, viz. the reacting or type-specific strain and the non-reacting or group strain. Each suspension gave a final titre in the water bath of 1 in 200 with a crude, *i.e.* untreated, Type 3 serum. Type 3 serum was then absorbed with each strain; the deposit of 100 c.c. of glucose broth culture contained in 1·25 c.c. was added to 0·1 c.c. of serum. Absorption with the group strain removed the agglutinin for the group strain, but failed to lower the titre of the serum for the type-specific strain. On the other hand, absorption with the type-specific strain removed the agglutinin (1 in 27 dilution tested) for both type-specific and group suspensions. This result has been repeatedly confirmed, and it has been found that, while a good type-specific colony culture will remove all the type-specific agglutinin after one treatment, four consecutive treatments, each with the deposit of 100 c.c. of the group strain, fail to affect the type-specific titre.

(*c*) *Appearances of the colonies on blood agar plates*

On blood agar there were no features which differentiated between type-specific and group colonies. The colonies might be either soft and translucent, or on very moist plates definitely watery or mucinous, in all cases with well marked clear areas around.

(*d*) *Stability of the serological characters of the group and type-specific colonies of Type* 3

(1) The effect of successive passages in broth of each serological variety was tested. At intervals plates were made from the broth subcultures and colony cultures were tested for agglutination. To give one example of numerous experiments, four colonies, one type-specific, No. 4, and three group, Nos. 5, 7 and 12, were subcultivated for nineteen generations in fluid media and finally plated; twelve colonies from each plate were tested with the following results.

Type-specific No.	4	colony culture plated	yielded	11	type-specific and		1	group
Group No.	5	,,	,,	4	,,	,,	8	,,
,, No.	7	,,	,,	3	,,	,,	9	,,
,, No.	12	,,	,,	0	,,	,,	12	,,

No. 12 colony culture remained apparently pure group, and a further plate from the twentieth generation in broth gave a similar result, that is to say, of twelve colonies none gave a type-specific reaction.

(2) The effect of passage through mice was tried. For this passage experiment the culture chosen was the No. 12 colony, which, as shown above, retained its group characters in culture. A suspension, consisting of the deposit of a mixture of 300 c.c. glucose phosphate broth and 100 c.c. of serum broth cultures, was injected subcutaneously into seven mice, each mouse receiving the deposit of 50 c.c. Plates were made from the blood of the mice and the agglutination of colonies was tested with the following results.

Mouse No.	Died	Result of examination of colony cultures
247	6 days	5 colonies = 1 group, 4 too granular
248	2 ,,	3 ,, = all type-specific
249	1 ,,	6 ,, = ,, ,,
250	2 ,,	3 ,, = ,, ,,
251	2 ,,	1 colony = group
252	2 ,,	3 colonies = all type-specific
254	2 ,,	18 ,, = 1 group, 17 type-specific

The test was repeated, using smaller doses of culture inoculated subcutaneously.

Mouse No.	Dose of culture deposit	Died	Result of examination of colonies
262	5 c.c.	2 days	4 colonies, all group
263	5 ,,	3 ,,	1 group, 3 type-specific
264	5 ,,	3 ,,	4 group colonies
265	10 ,,	5 ,,	12 group, 2 type-specific
268	20 ,,	1 ,,	2 group, 1 type-specific
270	50 ,,	2 ,,	4 group colonies

Further tests showed similar irregularity in the results, cultures from some mice giving evidence of reversion to the type-specific condition, those from others remaining group. For example, a mouse injected subcutaneously with 0·25 c.c. of No. 12 broth culture died in 2 days and yielded eleven group colonies and one type-specific; a second mouse dying in 3 days after a dose of 1·0 c.c. yielded all group colonies (twelve tested). The difficulty with regard to these experiments was the absence of any differences in appearance between the group and type-specific colonies on a blood agar plate, and it was impossible to be sure in instances where only group colonies were found that there had been no reversion to the type-specific condition.

(*e*) *Differentiation of type-specific and group colonies on a special medium*

Type 3 colonies, obtained on the primary plate composed of 5 per cent. horse blood agar sown with material from cases of acute infection, viz. scarlet fever, tonsillitis, etc., are almost invariably glistening (on the surface) and soft. On moist plates they tend to become watery or slightly mucinous. There was therefore no possibility of demonstrating the matt and glossy colonies described

by Todd, as from the outset all the colonies corresponded either to the glossy form or to the pseudo-glossy (watery or mucinous), to use the terms applied by that author. Moreover, as already stated, such colonies might be either type-specific or group. It was therefore interesting to find that on an agar medium, in which was incorporated a proportion of homologous agglutinating serum, these two serological varieties of Type 3 exhibited most striking differences in their colonial appearances.

In the preparation of the serum agar plates, in order to spare the serum, small plates measuring $2\frac{1}{2}$ in. in width were used, and a layer of plain nutrient agar was first allowed to set in the dish. This first layer was covered by a second composed of agar plus agglutinating serum, a sufficient amount being 4 c.c. containing about 2 per cent. of serum. Smaller quantities of serum down to 0·75 per cent. are sufficient to bring out the differential features of the colonies, but the higher percentages of serum make the medium more favourable for growth and the distinction more definite.

The agglutinating serum agar plate is spread with an actively growing subculture of Type 3 so as to obtain discrete colonies, and, after incubation overnight, is examined on a dissecting microscope by oblique substage illumination. On a plate made from a Type 3 strain which gives a good type-specific agglutination reaction, with the light suitably adjusted, colonies with the following appearances are seen: (1) uniformly opaque or coarsely stippled, (2) uniformly blue, translucent or finely stippled, and (3) partly translucent or partly opaque; those of (1) are rare, those of (2) occur rather more frequently, and the great majority fall into (3). The colonies when well spaced are circular in outline, and by reflected light show a slight central eminence and a glistening surface. In the mixed colonies the proportions of opaque and translucent growth vary. In some instances the opaque substance is represented by a tiny wedge-shaped patch in the margin, the remainder of the colony being translucent; in others it composes the greater part of the structure of the colonies, radiating from a dense centre like the spokes of a wheel. This radial arrangement is common, though sometimes a colony may be divided nearly equally into translucent and opaque sections. When touched with a platinum wire, differences in consistency between the two sections cannot be detected. No haloes have been seen around either variety of colony.

The appearance of Type 3 colonies is different on agar containing normal rabbit serum or immune sera prepared against heterologous types; there is no differentiation into clear and opaque areas, the substance appearing translucent and homogeneous.

(*f*) *Agglutination reactions of the opaque and translucent growths*

It is unfortunate that one cannot test streptococcal colonies directly against agglutinating serum, as is done in the work on the Salmonella phases, and that subcultures must first be made in a liquid medium. But such sub-

cultures in broth made from the opaque colony give emulsions agglutinating readily with the formation of coarse clumps on admixture with a purified Type 3 serum, while translucent colonies on the other hand give broth suspensions which generally remain unchanged, though occasionally there may be slight or moderate clumping, due, as will be shown later, to the development of cocci-producing type-specific substance, that is to say, to a partial reversion to the type-specific state. Thus it seems clear that the opaque colonies are type-specific and that the translucent are group, the presence of the antibody in the medium revealing the growth of organisms which are able to elaborate type-specific antigen.

If a perfectly translucent colony, *i.e.* a group colony, is subcultivated in broth, and the culture is plated on homologous agglutinating serum agar, the majority of the colonies produced after incubation overnight are translucent, while a few show varying amounts of opaque growth. On the other hand a plate made from an opaque, *i.e.* type-specific, colony culture generally presents much the same appearance as that described in detail above in the case of a Type 3 strain which gives good type-specific agglutination. The culture has become a mixture of type-specific and group elements, and on further subcultivation the tendency is towards loss of type-specific qualities. Complete loss is difficult to bring about artificially, as will be seen from the following passage experiment which was directed to that end. The experiment consisted in growing a Type 3 strain for many generations in broth and plating at intervals on a Type 3 agglutinating serum agar. Each subculture after the first was made from a plate colony, and care was taken to touch only the translucent substance of a colony. The plate was incubated for 3 days, so that the colonies might attain their maximum size, since it had been found that potential foci of type-specific growth within a colony might escape observation after 1 or even 2 days' incubation. In spite of this procedure, continued for twenty-six generations, the last plate from a broth culture of a translucent colony still showed an occasional opaque colony and many translucent colonies, which subsequently developed opaque foci in the margins. Even the tiniest opaque focus visible on the third day of incubation produced on transference to a fresh plate a majority of opaque colonies, which could be shown to be type-specific.

(*g*) *Mouse passage experiments*

Comparative virulence tests were made on mice with broth subcultures of opaque and translucent colonies of Type 3.

Dose	Method	No. of mice	Results: Type-specific cultures days	Results: Group cultures days
0·001 c.c.	Intrap.	8	4 died (1, 1, 7, 10)	3 died (1, 1, 3)
0·01 ,,	Intrap.	8	4 ,, (1, 1, 1, 5)	3 ,, (1, 1, 5)
0·01 ,,	Subcut.	8	2 ,, (14, 17)	1 ,, (13)
0·1 ,,	Subcut.	8	4 ,, (1, 1, 2, 4)	3 ,, (1, 4, 5)

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In this experiment there was a little evidence pointing to the type-specific culture being the more virulent, since two out of sixteen mice survived the inoculation of type-specific culture, while six out of sixteen survived the group inoculation (the surviving eight mice were killed in 21 to 31 days, and the spleens gave negative cultures). From seven mice, inoculated with group culture, plates of Type 3 serum agar were sown directly from the blood or spleen; the cultures which grew were mixtures of opaque and translucent colonies, the latter predominating. Plate cultures were also made from mice injected with opaque colony cultures; the blood from one mouse dying within 24 hours yielded nearly all opaque colonies, but two mice dying in five and seventeen days respectively yielded a majority of group colonies. Residence in the mouse's tissues does not consistently stimulate the production of type-specific substance in a group culture, and may apparently cause a type-specific culture to throw off group colonies.

The following are some instances where plating on Type 3 serum agar has been helpful in settling the identity of a doubtful strain. (1) A scarlatinal strain, S.F. 15, which was very granular in early subcultures gave a doubtful Type 3 reaction. It was subcultivated for many generations and finally gave a uniform suspension which failed to agglutinate with Type 3. An agglutinating serum was prepared from it, and this agglutinated the homologous S.F. 15, but not Type 3. For some time it remained doubtful whether the strain was a new type, but after plating it on S.F. 15 serum agar and on Type 3 serum agar the position became clear. On the homologous serum agar all the colonies were uniformly translucent, while on the type-specific Type 3 serum agar not all were quite translucent, some few exceptions showing opaque patches. Subcultures were made from the opaque patches, and these gave typical coarse flocculation when tested with purified Type 3 serum. The explanation was that the strain S.F. 15 was a Type 3 which had become group in character after subcultivation, and the serum prepared with it in the rabbit was lacking in type-specific agglutinins. (2) My original Type 3 was a strain 55/4 obtained from Dr Zingher, New York Health Department, in 1925. In 1934, Dr Anna Williams sent me this same strain, which had been kept in cultivation in America. When tested versus a purified Type 3 serum it failed to agglutinate. A plate was therefore made on Type 3 serum agar, and this showed translucent colonies, excepting a few with small opaque foci. From these opaque foci opaque colonies were obtained which on growth in broth gave good Type 3 agglutination. (3) S. 84, one of Dochez, Avery and Lancefield's classical strains (1919), was given to me by Dr Todd in 1934. This culture, which was virulent for mice and produced watery colonies on blood agar, gave a doubtful agglutination reaction by the slide method with a purified Type 3 serum. The culture was accordingly plated on Type 3 serum agar, and it was found that the great majority of colonies were translucent and a few only showed opaque foci. An opaque focus was plated directly on a fresh Type 3 serum agar plate and produced a mixture of translucent and

opaque colonies; one of each variety was grown in broth and tested versus a purified Type 3 serum. The opaque colony culture gave immediate coarse flocculation and was undoubtedly type-specific; the translucent colony gave a doubtful trace of agglutination, *i.e.* it was a group colony. (4) Three cultures were sent to me, two from children with scarlet fever and one from a nurse who was suspected on epidemiological grounds of having communicated the scarlatinal infection to the children. The strains from the two children were typical examples of Type 3, but the nurse's strain could not be identified with certainty. In view of the circumstances the culture from the nurse was plated on Type 3 serum agar. The majority of the colonies produced were translucent, but a few showed opaque foci from which a type-specific colony of Type 3 was obtained. Thus the suspicion that the nurse had carried the scarlatinal infection was strengthened.

In America, Type 3 strains appear to be of common occurrence in scarlatina and other conditions. In 1932, Miss A. R. Beebe, Yale University, sent me a number of strains which she had identified as Type 3. These strains, like the Type 3 strains in this country, produced on blood agar shiny, soft, translucent, usually conical colonies, excepting one (Granberg, otitis media) where the colonies were slightly coherent and irregular in outline. On Type 3 agglutinating serum agar 8 out of 12 strains produced a mixture of colonies with three grades of density, viz. (1) very opaque colonies, (2) opaque colonies, and (3) translucent colonies; in four cultures the very opaque colonies were not present. In trypsinised meat broth the very opaque colonies formed coarsely granular growths which could not be tested for agglutination; the opaque colonies gave typical flocculation with purified (type-specific) Type 3 serum, while the translucent colonies gave either no agglutination or a mere trace. When the "very opaque" colonies were subcultivated in successive broth cultures they gradually grew more uniformly, and when plated on agglutinating serum agar formed only opaque and translucent colonies.

(*h*) *Colonial differentiation of types other than Type* 3

This has been studied on homologous agglutinating serum agar, but so far no other type has been found to give such definite results. (*a*) Type 1 on homologous agglutinating serum agar yielded colonies, all of which showed opaque, stippled centres and translucent margins. All colonies grown in plain broth gave type-specific agglutination, but slight differences could be detected between individual colonies when grown in ascites broth, and even between different parts of the same colony. For example, the extreme margin of a colony grown in ascites broth gave no agglutination on the slide with Type 1 serum, while the ascites broth subculture from the centre of the same colony agglutinated well. (*b*) Type 2 produced on homologous serum agar colonies differing slightly in opacity when viewed by transmitted light, but the differentiation was less marked than in the case of Type 3 strains. There were

also slight differences in agglutinability; the more opaque colonies grown in broth, when tested against a specific Type 2 serum, gave the immediate coarse flocculation characteristic of a freshly isolated Type 2 strain, while the less opaque colonies reacted much more slowly and the clumps were loose and could be readily dispersed. The above results are consistent with the fact that Types 1 and 2 have retained their serological type characters in culture with very little noticeable change for many years.

The following two types on the other hand readily lose type-specificity, but the differentiation between type-specific and group colonies is not clear on account of the very fluid consistency of the colonies. (*c*) S.F. 73/4 (Type 19), which had been long in cultivation, was plated on horse blood agar and produced watery or slightly mucinous colonies. Colonies were picked off and grown in broth, and the broth cultures were tested for agglutination against homologous purified type serum; out of thirty-four colony cultures two agglutinated well, one gave a trace and the rest were negative. On homologous serum agar S.F. 73/4 formed large spreading watery colonies, opaque in the centre with thin translucent margins, in which could be detected ill-defined slightly more opaque patches. Subcultures of the opaque and translucent patches showed that the former were generally though not invariably type-specific, reacting on the addition of purified homologous serum with the formation of coarse clumps; cultures of the translucent patches failed to agglutinate with the type-specific serum. The type-specific colony culture readily removed the homologous agglutinin from S.F. 73/4 serum, while the non-agglutinating culture failed to exhaust the serum even after three treatments each with the deposit of 100 c.c. of glucose broth culture. The stability of the serological characters of the colony cultures was tested as follows. Two type-specific colony cultures and two group colony cultures were plated, and twelve colonies from each were grown in broth; the agglutination results were as follows:

Type-specific colony 1:	11	colonies	were	type-specific,	1	was	group
„ „ 2:	12	„		„			
Group colony 1:	1	colony	was	type-specific,	11	were	group
„ „ 2:	1	„		„	11	„	

(*d*) Type 13 produced large spreading watery colonies on homologous serum agar with very ill-defined differentiation into translucent and opaque patches. There were marked differences in agglutinability between different parts of the same colonies, but it could not be determined whether these corresponded with the variations in opacity. From both Type 13 and 73/4 (Type 19) the agglutinating sera prepared in rabbits with opaque colony cultures were more type-specific than the original sera prepared from the stock strains.

(*i*) *Appearance of haloes in the medium around colonies*

When viewed by oblique transmitted light, opalescent haloes were occasionally seen in the clear serum agar surrounding the colonies. Some types produced haloes on homologous agglutinating serum as well as on heterologous

sera, and halo production could not be correlated with type-specificity of colonies. The majority of type strains did not produce haloes, and they were never seen, as already mentioned, around either the opaque or the translucent colonies of Type 3. The reason for their appearance has not been determined. It may depend on the varying vigour of acid production in the medium.

VI. Epidemiological observations

A brief reference has been given under the heading of each type to the outbreaks of disease in which that type has been the causal organism. The following accounts, partly in tabular form, give the sequence of events in a few selected residential schools, where during each term for a number of years throat swabs have been taken from pupils admitted to the school sanatorium on account of nose or throat troubles. No systematic attempt was made to discover the distribution of haemolytic streptococci amongst the healthy residents, and the observations on carriers were in general confined to those who were suspected to have some catarrhal infection of the upper air passages; it will be seen from the column headed "swabs with no haemolytic streptococci" that a considerable number of such cases yielded negative swabs.

The finding of haemolytic streptococci of the pyogenes group in a throat swab does not prove that they were responsible for any disease which might be present, and one is frequently at a loss to say whether the carrier might possibly be a source of danger to other people. In the first place the numbers of colonies obtained must be considered, and some may take the view with, I think, considerable justification, that the presence in the throat of numerous haemolytic streptococci, practically replacing the ordinary flora, is evidence of a present or recent acute streptococcal infection. Apart from obvious disease conditions which account for such bacteriological results, as, for example, follicular tonsillitis and scarlet fever, the possible significance of a positive swab can only be determined if one knows the bacteriological history of the carrier and of the community in which he moves, as well as the serological identity of the streptococcal strains found in each instance. For instance, in the following schools, where to some extent the above information has been available, it has been possible to conclude in the cases of two boys with a few haemolytic streptococci in their throats that one was the subject of a transient infection with the prevailing type, and that the other was still carrying the type which had caused his attack of tonsillitis in a previous term. But one must admit that at present there are no means of affirming that a carrier will set up disease.

Streptococcal history of schools, etc.

School D/B. This school is a residential school of about 330 boys. Throat swabs were examined from cases suffering from catarrhal infections of the upper air passages, and from 1931 to 1934 practically all patients admitted to the sanatorium excepting surgical casualties were swabbed, and the swabs

examined for the presence of colonies of haemolytic streptococci. Table I shows the appearance of epidemic types, as well as the occurrence of sporadic infections, and their persistence during subsequent terms.

In the first term of the investigation an outbreak of infection with Type 17 occurred at the school in December, 1929, and profuse cultures were obtained

Table I

erm and Year		Types of *Str. pyogenes* occurring in primary epidemic waves	Types of *Str. pyogenes* occurring in secondary waves and sporadically	Unidentified *Str. pyogenes*	Swabs with no haemolytic streptococci
inter	1929	Type 17. 3 cases of otitis media	—	6	—
nt	1930	None	Type 17. 9 cases; 4 otitis, 1 with meningitis; 5 tonsillitis and rheumatism	—	—
mmer	,,	,,	—	—	—
inter	,,	,,	,, 17. 2 fresh infections	6	12
nt	1931	,, 18. 63 cases; febrile sore throat; no rash, no otitis	,, 17. 1 fresh infection	8	41
mmer	,,	None	,, 18. 1 fresh infection ,, 18. 3 carriers found	5	39
inter	,,	,, 13. 8 cases of tonsillitis	,, 1. 2 cases (1 scarlet fever) ,, 7. 2 cases ,, 4. 1 case	14 (sparse colonies, 1 or 2 to a few)	35
nt	1932	*? Type X. 112 cases; tonsillitis, pyrexial colds, pneumonia, otorrhoea	,, 13. 30 cases; tonsillitis and sore throats	—	63
mmer	,,	None	,, 13. 4 fresh infections	3	5
inter	,,	,,	,, 13. 8 fresh infections ,, 13. 7 carriers found ? Type X. 13 cases	16 sparse carriers	101
nt	1933	Type 1. 40 cases; tonsillitis; one mastoid with rash	Type 13. 2 fresh infections ,, 13. 9 carriers (4 former cases) Types 4, 7. Each 1 carrier	13 sparse carriers 2 acute infections	96
mmer	,,	None	Type 13. 1 carrier found ,, 1. 2 carriers found ,, 27. 2 carriers found	6 sparse carriers 2 acute infections	42
inter	,,	,,	,, 13. 2 carriers found ,, 1. 2 carriers found ,, 7. 2 carriers found	2 sparse carriers	47
nt	1934	,, 6. 18 cases; tonsillitis and otorrhoea (3)	,, 13. 3 carriers found ,, 3. 2 infections (1 scarlet fever)	4 sparse carriers 2 acute infections	43
mmer	,,	None	,, 6. 3 fresh infections ,, 6. 1 carrier found	2 sparse carriers 1 acute infection	9

* This type was not identified serologically but, judged by cultural appearances, the majority of strains designated ? Type X were probably of the same type.

from the throats and ear discharges of three boys. During the next school term, from 27th February to 2nd April, 1930, Type 17 was obtained from the ear discharges of four cases of otitis media, one of which developed mastoid disease followed by meningitis (Type 17 was grown from the cerebro-spinal fluid), and five cases of tonsillitis which developed symptoms of acute rheu-

matism, viz. effusion of joints and carditis. There were no scarlatiniform rashes. In the Winter term, 1930, Type 17 was obtained from two cases of pyrexial sore throat, and in the Lent term, 1931, from a boy with sore throat without fever. Up to the end of Summer, 1934, Type 17 has not again been found in any swabs from this school.

Type 13 appeared in the Winter term, 1931, and there were eight cases of tonsillitis, the majority of the follicular type, from which profuse cultures were obtained. In the Lent term, 1932, there was an extensive outbreak of feverish colds and tonsillitis, partly of pneumococcal or of unknown origin and partly streptococcal. There were thirty cases from which Type 13 was recovered (mainly tonsillitis and follicular tonsillitis, one case of herpes, and one of acute rheumatism with pneumonia). There were also three boys who suffered from acute infection in the previous term still carrying Type 13. (During this term there were many cases, approximately 112, from which a different type of streptococcus was obtained, a type which could not be identified with the sera then available. In the absence of serological identification one cannot be certain that all the strains were of the same type, but they all formed opaque colonies and granular suspensions, differing sharply from Type 13 which formed clear watery colonies and uniform suspensions. There were seven boys who at different times during the term contracted acute infections with both types, first with one then with the other.) In the Summer term, 1932, there were four fresh cases of sore throat which yielded profuse cultures of Type 13. In the Winter term, 1932, Type 13 was obtained from fifteen boys, five of whom were carriers (one having been an acute case in the Winter, 1931, and four in Lent, 1932). It is of some interest that there were also four sparse carriers of haemolytic streptococci which could not be typed, who had been Type 13 cases in Lent and Summer, 1932. It is not impossible that these carrier strains were originally Type 13 and had lost their type-specific characters. In Lent, 1933, there were two fresh acute cases, and nine carriers found (four of whom were cases in Lent, 1932). In Winter, 1933, two carriers were found who had their acute infections in the Lent term, 1932. In the Lent term, 1934, there were three sparse carriers, two of whom were infected in Lent, 1932, and Lent, 1933, respectively. It seems highly probable that the strain of Type 13 had persisted in the school since its first appearance in Winter, 1931. In the Summer term, 1934, no carrier of Type 13 was found, but only few (17) swabs were examined.

Type 18 appeared in the Lent term, 1931. It was obtained from throat swabs, almost invariably in profuse culture, from sixty-three boys who were suffering from some form of inflammation of the throat with fever; no rashes were observed and no cases of otitis media were reported. During the Summer term, 1931, when swabs were examined from every case of pyrexia and sore throat, Type 18 was obtained from four boys only; one, a case of follicular tonsillitis, yielded a profuse culture, whilst the colonies were sparse from the other three boys, two of whom had suffered from an acute infection with this

type during the preceding term. Type 18 has not reappeared in this school up to the end of the Summer term, 1934.

Type 1. This type appeared in the school about the middle of the Lent term, 1933. From 16th January to 15th February 100 swabs were examined from cases admitted to the sanatorium; twenty-eight yielded *Str. pyogenes* of various types, none being Type 1, and seventy-two were negative with regard to the presence of haemolytic streptococci. On 16th February there was a case of sore throat which yielded numerous colonies of Type 1, and from that date until the end of the term there were forty cases of Type 1 infection, including three of the nursing staff. All the cases were tonsillitis or follicular tonsillitis; there was only one case which developed a rash following infection of the mastoid; the complications were few—including acute rheumatism, septic herpes zoster and erysipelas of the face. Two boys were still carrying Type 1 in the following June, 2 months from the date of the acute attack. One of them was still a carrier in November, 1933, and in December, 1933, another Type 1 carrier was found the date of whose acute infection was March, 1933.

Type 6. This type appeared in the school in the middle of the Lent term, 1934. Prior to 1st March, when the first culture was obtained from a case of tonsillitis, forty-seven swabs had been examined since the term began on 25th January, and there was no evidence of its presence. Until the end of the term on 3rd April infection with Type 6 produced eighteen cases of sore throat or follicular tonsillitis, followed in two cases by otitis media and in one by antral disease; a typical scarlatinal rash was reported in one case only. Excepting two sparse carriers of one unidentified type and one Type 13, Type 6 accounted for all the streptococcal infections in the sanatorium during the period of its prevalence. In the Summer term, 1934, there were two acute cases of follicular tonsillitis which yielded abundant cultures of Type 6, and one case which yielded a sparse culture. One boy, infected the previous term, was still a sparse carrier.

School R/B. It is unnecessary to analyse Table II in detail, as the sequence of events is similar to that in the preceding school. There were three small outbreaks of scarlet fever due to Type 3 and Type 11 (twice) which appeared to be promptly checked, whereas the Type 2 outbreak in Winter, 1931, in which there were no definite scarlatinal cases, was much more extensive and the infection appeared to linger in the school for more than two years. In the Winter term, 1933, there was no new epidemic strain introduced and no extensive outbreak of infection, although there was present in the school a considerable variety of types, six of which had been responsible for outbreaks during the preceding terms. It will be observed that during the three years seventeen different types had been identified.

School Q.B. At School Q.B., Table III, the outbreak in Lent, 1931, was extensive and there were some severe cases of illness. There is little doubt that the infections were mainly due to Type 1, which was the type of streptococcus obtained from the nine cases examined. The infection appeared to persist in

Table II

Term and Year	Types of *Str. pyogenes* occurring in primary epidemic waves	Types of *Str. pyogenes* occurring in secondary waves and sporadically	Unidentified *Str. pyogenes*	Swabs with no haemolytic streptococci
Lent 1931	Type 11. 5 cases; 3 scarlet fever, 2 tonsillitis	—	3	20
Summer ,,	—	Type 11. 1 carrier	5 (acute tonsillitis)	—
Winter ,,	,, 3. 7 cases; 5 scarlet fever, 2 tonsillitis, one followed by otitis ,, 2. 45 cases; pyrexial sore throat, one doubtful rash	,, 5. 1 sore throat	6	36
Lent 1932	—	,, 2. 5 fresh throat infections ,, 2. 6 carriers (cases last term) ,, 3. 2 cases; 1 naso-pharyngeal catarrh, 1 ? scarlet fever ,, 5. 1 carrier (case last term) ,, 7. 1 tonsillitis	12	55
Summer ,,	,, 7. 3 cases of acute tonsillitis	,, 2. 3 fresh cases of tonsillitis ,, 2. 1 carrier (case in Dec. 1931)	—	12
Winter ,,	,, 9. 26 cases; tonsillitis, simple and follicular; no rashes	,, 2. 3 fresh throat infections ,, 6. 1 case of tonsillitis	11 (4 in abundant culture)	32
Lent 1933	,, 11. 4 cases of scarlet fever ,, 5. 3 cases of tonsillitis	,, 9. 1 fresh case of tonsillitis ,, 9. 2 carriers (cases last term)	1	22
Summer ,,	—	,, 2. 1 case of tonsillitis ,, 5. 1 case of tonsillitis ,, 15. 1 case acute antral disease	1	11
Winter ,,	—	,, 2. 1 fresh case of tonsillitis ,, 2. 1 carrier (case in Oct. 1931) ,, 3. 1 case of tonsillitis ,, 4. 1 otitis media, 1 carrier ,, 5. 1 carrier (case in Lent 1933) ,, 7. 1 case of tonsillitis ,, 9. 1 case of tonsillitis ,, 11. 1 carrier (case in Lent, 1933) ,, 16. 1 case, ulcer on fauces	3	38
Lent 1934	,, 25. 4 cases of tonsillitis ,, 12. 6 cases of tonsillitis	,, 13. Throat of measles case ,, 5. 5 cases of acute tonsillitis	1	21
Summer ,,	—	,, 1. 1 case of tonsillitis ,, 5. 6 cases; 3 simple and 3 follicular tonsillitis ,, 12. 9 cases; 6 tonsillitis, 3 abundant throat cultures in *rubella* ,, 16. 1 case of tonsillitis ,, 25. 1 case of tonsillitis	1	24

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Table III

Term and Year	Types of *Str. pyogenes* occurring in primary epidemic waves	Types of *Str. pyogenes* occurring in secondary waves and sporadically	Unidentified *Str. pyogenes*	Swabs with no haemolytic streptococci
Lent 1930	Type 6. 3 cases of tonsillitis	—	4	5
Summer ,,	—	Type 6. 11 cases; pharyngitis, tonsillitis	4	4
Lent 1931	,, 1. ?number; 9 cases examined, tonsillitis naso-pharyngitis, otitis; some doubtful rashes	—	3	4
Summer ,,	—	,, 1. 3 cases; pharyngitis, post-nasal catarrh	3	10
Winter ,,	—	,, 1. 1 fresh case of tonsillitis ,, 1. 2 carriers (cases in March and July, 1931) ,, 16. 1 case of pharyngitis	—	6
Lent 1932	—	—	—	10
Summer ,,	—	—	—	5
Winter ,,	,, 13. 21 cases; tonsillitis, post-nasal catarrh and coryza	,, 1. 1 fresh infection (otitis media) ,, 2. 2 cases; post-nasal catarrh, tonsillitis	14	17
Lent 1933	—	,, 22. 1 case of tonsillitis	—	3
Summer ,,	,, 15. 14 cases; tonsillitis, followed by otitis media 1	,, 13. 1 fresh case of tonsillitis (developed scarlatina 3 weeks later due to Type 1 and still carrying Type 13) ,, 1. 1 case of acute tonsillitis	1	13
Winter ,,	—	,, 13. (1 carrier in case which had Type 1 scarlet fever last term)	2	4
Lent 1934	,, 5. 3 cases of tonsillitis, 2 of which developed otitis media ,, 11. 3 cases; 1 tonsillitis, 2 scarlet fever, one of which developed otitis media	,, 15. 1 carrier, sparse colonies ,, 22. 1 case of tonsillitis	5	45
Summer ,,	—	,, 7. 1 sore throat ,, 12. 1 case of pharyngitis ,, 13. 3 fresh cases of tonsillitis ,, 13. 1 carrier, sparse colonies ,, 22. 1 fresh case of tonsillitis ,, 22. 1 carrier (case last term)	—	19

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the school until Summer, 1933, although one cannot exclude the possibility of another strain of Type 1 having been introduced. Type 13 again showed itself to be a very persistent source of infection, as at School D/B.

School F.B. In only two instances, viz. at School D.B. and at this school, Table IV, has Type 18 been found in outbreaks, and it is interesting that both outbreaks occurred in Lent, 1931. The two schools are widely separated, and it is unlikely that the infection was conveyed from one to the other.

Table IV

Term and Year	Types of *Str. pyogenes* occurring in primary epidemic waves	Types of *Str. pyogenes* occurring in secondary waves and sporadically	Unidentified *Str. pyogenes*	Swabs with no haemolytic streptococci
Winter 1930	Type 18. 27 cases; simple and follicular tonsillitis, 1 otitis media	—	1	—
Lent 1931	—	Type 18. 27 fresh cases ,, 18. 2 carriers (cases last term)	4	7
Summer ,,	,, 5. 2 cases of otitis media	,, 18. 4 fresh cases ,, 18. 2 carriers (cases previous term)	3	44
Winter ,,	—	—	1	8
Lent 1932	—	—	2	3
Summer ,,	—	,, 1. 1 case of otitis media	—	4
Winter ,,	—	,, 1. 1 case of tonsillitis	—	—
Lent 1933	,, 6. 17 cases; tonsillitis and rhinitis, complicated by otitis media 4, impetigo 2, lobar pneumonia 1	,, 1. 2 cases; tonsillitis, pharyngitis, with adenitis	—	5
Summer ,,	—	,, 6. 5 fresh cases; nasopharyngitis, tonsillitis and 2 otitis media	3	4
Winter ,,	,, 12. 2 cases of tonsillitis	—	—	2
Lent 1934	,, 14. 8 cases; acute sinusitis, tonsillitis, with 2 otitis media ,, 22. 6 cases of tonsillitis	—	—	12
Summer ,,	—	,, 6. 1 case of follicular tonsillitis ,, 12. 2 cases of tonsillitis ,, 14. 17 cases; tonsillitis simple and follicular 14, nasopharyngitis 3, followed by otitis media 1 ,, 16. 1 case of tonsillitis	4	7

School J.B. At School J.B., Table V, in Lent, 1931, there was an outbreak of infection due to Type 1, each of the fourteen cases being typical scarlet fever. At the beginning of the next term eight of the convalescents were found still to be carrying Type 1 streptococcus, but only two further cases of Type 1 infection occurred. In the Winter, 1933, the Type 1 infection was probably due to a different and less toxigenic strain of Type 1, only two of the four cases

being typical scarlet fever. An outbreak of typical scarlet fever (five cases) due to Type 2 was apparently promptly checked in the Summer term, 1933.

School U.B. Only two observations have been made at School U.B., Table VI, one in Lent and one in Summer, 1934, but it is known that for some time there had been a high incidence of throat infections. This fact no doubt accounts for the diversity of types of *Str. pyogenes* which has been found.

Table V

Term and Year	Types of *Str. pyogenes* occurring in primary epidemic waves	Types of *Str. pyogenes* occurring in secondary waves and sporadically	Unidentified *Str. pyogenes*	Swabs with no haemolytic streptococci
Lent 1931	Type 1. 14 cases of scarlet fever	—	—	—
Summer ,,	—	Type 1. 2 cases; scarlet fever, tonsillitis ,, 1. 8 carriers (cases last term)	—	6
Winter ,,	—	,, 5. 1 case of tonsillitis	—	2
Winter 1932	—	—	1 (scarlet fever)	—
Lent 1933	—	—	1 (? scarlet fever)	6
Summer ,,	,, 2. 5 cases of scarlet fever, 1 developed otitis media	,, 12. 1 case of tonsillitis	3	7
Winter ,,	,, 1. 4 cases; 2 scarlet fever, 2 sore throats, 1 developing mastoid disease	,, 9. 1 case of tonsillitis ,, 13. 1 sore throat ,, 17. 1 otitis media	1	9
Lent 1934	—	,, 1. 1 otitis media ,, 22. 1 case of tonsillitis	—	3
Summer ,,	—	,, 12. 1 case of pyrexia	—	1

Table VI

Term and Year	Types of *Str. pyogenes* occurring in primary epidemic waves	Types of *Str. pyogenes* occurring in secondary waves and sporadically	Unidentified *Str. pyogenes*	Swabs with no haemolytic streptococci
Lent 1934	Type 1. 3 cases; 2 tonsillitis, 1 otitis media ,, 3. 8 cases; 3 scarlet fever, 3 tonsillitis, 2 otitis media ,, 6. 3 cases; otitis 1, scarlet fever 1, tonsillitis 1	Type 8. 1 case of scarlet fever ,, 14. 1 case of otitis media ,, 27. 1 case of scarlet fever	2	5
Summer ,,	—	,, 1. 2 cases of tonsillitis ,, 3. 3 cases; 2 tonsillitis, 1 otitis media ,, 5. 1 case of tonsillitis ,, 25. 2 cases of tonsillitis	—	—

A few further examples are given of observations made on the serological types of streptococci occurring in outbreaks in institutions. They furnish additional evidence of the epidemiological importance of certain types. (1) From June, 1932, to the end of February, 1933, at a large country hospital for convalescent rheumatic children swabs were taken from cases of pyrexial sore throat, and the strains of streptococci obtained were studied serologically.

The following is a list of the types with the number of occasions on which each was found: Type 13, 13 cases; Type 5, 5 cases; Type 14, 4 cases; Type 17, 3 cases; "Sylvia Turton," 3 cases; Type 9, 2 cases; Type 22, 2 cases; Type 8, 2 cases; Type 1, 1 case; Type 2, 2 cases. The multiple cases due to one type usually occurred in the same ward. (2) At an orthopaedic hospital for children, from January, 1932, to October, 1932, the following small outbreaks of scarlet fever or sore throat occurred in succession: Type 2, 5 cases; Type 1, 6 cases; Type 5, 6 cases; Type 1, 4 cases; Type 13, 1 case; Type 22, 2 cases; Type 3, 3 cases. (3) At a general hospital in London where there had been a prevalence of scarlet fever, swabs were examined during March and April, 1933. The prevailing scarlatinal infection was due to Type 4, which was found in thirty-one cases. Other types occurring in scarlet fever and tonsillitis cases were: Type 1, 5 cases; Type 5, 3 cases; Type 2, 1 case; Type 3, 1 case; Type 19, 1 case; Type 12, 1 case; Type 27, 1 case. (4) An outbreak of scarlet fever due to Type 2 occurred in 1931 at a children's sanatorium in Northumberland, the vehicle of infection probably being raw milk. Out of 310 resident patients 170 contracted scarlet fever, 133 of the cases occurring within 3 days. Type 2 was obtained from the throats of the fifteen scarlatinal patients examined, and also from the throats of four inmates and four members of the staff who did not contract clinical scarlet fever. (5) The serious milk outbreak of streptococcal tonsillitis during November and December, 1929, in Brighton and Hove, is reported in the Chief Medical Officer's *Annual Report* for 1930. The type of streptococcus concerned was Type 9. (6) The following types of *Str. pyogenes* were obtained from the purulent discharges of patients with otitis media or mastoid disease during the period from January, 1930, to April, 1934. The following types were found out of a total of 78 cases: Type 1, 9 cases; Type 2, 2 cases; Type 3, 3 cases; Type 5, 11 cases; Type 6, 17 cases; Type 11, 1 case; Type 12, 5 cases; Type 13, 1 case; Type 14, 2 cases; Type 15, 2 cases; Type 17, 5 cases; Type 18, 1 case; unidentified, 19 cases.

(7) At School X, a preparatory boarding school of sixty-five boys, there was an outbreak of acute throat infections in October-November, 1931. The *Str. pyogenes*, Type 13 ("Carter"), was obtained from almost all the cases. The clinical manifestations of this single infection took various forms, viz. severe tonsillitis (eight boys and five members of the staff) with high pyrexia and adenitis, otitis media and mastoiditis (3 cases), broncho-pneumonia (2 cases). There was one case of erysipelas of a wound, but this was not examined bacteriologically. A few apparently healthy carriers of Type 13 were found. In the Lent term, 1932, fifty-seven boys returned to school, and there occurred forty-four cases of influenza within a fortnight of the beginning of the term. On February 20th, a swab was received from the ear of a boy who had suffered from influenza, mild sore throat, acute otitis media, basal broncho-pneumonia and double maxillary sinusitis; a profuse culture of the *Str. pyogenes*, Type 5 ("Franklin"), was obtained. On March 3rd, thirty-five throat swabs were examined; twenty were from convalescent boys and

fourteen of these yielded cultures of streptococcus Type 5; of five swabs from the nursing staff four, all of whom had suffered from tonsillitis, were carrying Type 5; among ten of the teaching and domestic staff there was one carrier of Type 5 and one of Type 13, the prevalent strain of the preceding term. There was also one carrier of this latter type among the boys. The complications of the acute influenzal attacks were otitis media (10 cases), mastoid operation (7 cases), scarlatinal rashes (3 cases), broncho-pneumonia (6 cases). Two boys who were the subjects of acute infection with Type 13 in the preceding term contracted the Type 5 infection. In the Summer term, 1932, there was one fresh case of throat infection with Type 5.

(8) At School Y (a preparatory school), during the Summer term, 1931, an outbreak of streptococcal infections of the upper air passages occurred, which was of special interest on account of the particular type of *Str. pyogenes* concerned. This was Type 2, which is one of the two types most often associated with scarlet fever in epidemic form in this country. The clinical conditions, however, which included naso-pharyngitis, tonsillitis and sore throats, all with high fever, were characterised by the absence of anything of the nature of a scarlatinal rash. It was highly improbable, though this point was not actually tested, that all the subjects attacked, seventeen in number and the majority young children, were Dick-immune. The alternative hypothesis that the absence of rashes was attributable to deficiency on the part of the infecting strain in the production of Dick toxin was confirmed by intradermal tests with filtered broth culture on susceptible subjects. Dr W. Gunn, who kindly made the tests for me, reported that the reaction produced by the intradermal injection of a 1 in 50 dilution of broth culture of one strain was little greater—12 mm. compared with 10 mm.—than that following the injection of a 1 in 1000 dilution of the standard Dochez toxin. Broth cultures from two other strains contained even less Dick toxin, and only slight skin reactions were induced when injected neat. The infection was in several instances very persistent and four of the boys were still carrying the Type 2 strain 7 to 12 weeks after the initial attack. In the Summer term, 1932, there was an outbreak of throat infections, not all of which were examined bacteriologically, and throat swabs from four cases, including one with discharging ears, yielded numerous colonies of streptococcus Type 5.

The observations which are summarised above obviously raise questions of epidemiological importance which cannot be fully discussed here. In School D/B (Table I), for example, in four years six separate invasions with *Str. pyogenes*, each consisting of throat infections (and their attendant complications), were identified. Each invasion appeared suddenly, as far as can be seen, and after producing cases of disease in varying number and severity, gradually died out, leaving trace of its occurrence in the shape of chronic carriers. The question is, does the disappearance of the streptococcus mean that all or nearly all the persons susceptible to infection with this particular type had either been attacked, or had become insusceptible as the result of

manifest or concealed infection, or does it mean that the particular streptococcus strain had lost its invasive properties, and, though still present (in the chronic carriers), was no longer able to infect and produce disease in normal throats? The duration of a particular infection in a community undoubtedly depends upon the measures taken to prevent its spread, and it is noteworthy that outbreaks due to rash-producing strains are often more promptly checked than those in which the clinical manifestations are sore throat and tonsillitis. In the disappearance of an infection several factors are probably concerned, but the relatively low infectivity of streptococcal carriers among scarlatinal convalescents points to some change in the organism. It is possible that, in order to remain highly infective, the streptococcus must be exchanged from one susceptible throat during the throat's period of acute inflammation to another, and when such throats are no longer available the streptococci, now surviving only in convalescent (refractory) throats, lose their infectivity. One might suggest, moreover, that the type-specific quality of a strain which, as has been shown, can diminish and almost disappear in subculture, may do the same in the infected throat, its diminution or disappearance there involving loss of infective capacity. I have mentioned elsewhere that a streptococcus strain occurring in small numbers in a normal throat is generally more difficult to type than an epidemic strain, and it has been noted also that patients who have had an acute infection with a known type may months afterwards be carriers of a streptococcus which cannot be identified serologically. Furthermore, these observations show definitely that infections with the different streptococcal types represent different epidemic entities, and that infection with one leaves no immunity to another or at least no complete immunity.

VII. Discussion

It is clear both from the cultural and the immunological evidence that the haemolytic streptococci which are pathogenic to man and give rise to tonsillitis, scarlet fever, septicaemia and a variety of other clinical conditions constitute a well-defined bacterial species, for which the designation *Str. pyogenes* seems now to be generally accepted. Like the pneumococcus the *Str. pyogenes* has become differentiated into a number of serological races, which have to some extent different pathological and epidemiological values. As regards the stability of these races, there is no proof of any rapid transformation under natural conditions which would lessen their epidemiological significance, and in artificial cultivation for many years they have maintained their serological individuality. They are, however, subject to certain changes which affect their type-specificity, that is to say, the character or function upon which their serological identification depends, and it is to this circumstance combined with special technical difficulties of agglutination work with them that much of the past confusion and uncertainty can be attributed.

The loss of type-specificity which is responsible for such pronounced variations in the serological behaviour of *Str. pyogenes* is a phenomenon

observed in the serology of other bacterial species which differ widely from *Str. pyogenes* in morphology, antigenic composition and pathogenic action. It may be useful to discuss some of these species, and to see how far their behaviour is analogous, and how far it differs in mechanism. The pneumococcus may first be considered, since its maintenance in the type-specific state with which virulence is closely correlated is of the highest importance in the study of its immunity reactions. This importance was recognised at an early stage in serological work on pneumococci (Neufeld), since they or their products when obtained from the peritoneal cavity of an inoculated mouse were particularly suitable for rapid and accurate diagnosis of type. When culture suspensions were used results were not always satisfactory; the definition of type was less sharp, and to some observers it appeared necessary to assume the existence of subtypes. This confusion was cleared up by the demonstration that loss of virulence, which was difficult to avoid in cultures of pneumococci on artificial media, entailed a modification of the antigenic structure. It was found that avirulent strains had antigenic properties in common no matter from what type they originated; that is to say, avirulent antigen was characteristic of the whole pneumococcus group, while virulent antigen was specific for each pneumococcal type.

The modification in antigenic properties which the virulent pneumococcus had undergone in assuming the avirulent form consisted in the loss of the power to produce type-specific carbohydrate (incidently the absence of this secretion caused the pneumococcus to assume a more compact, coherent colony form, the so-called R form). Although this was simply a dérangement of a function, which was recovered spontaneously in some cases, and in others could be restored by animal passage, its effect was the appearance of a variant which could not be identified with the parent strain by the usual serological tests.

A serum prepared with this avirulent variant did not agglutinate the virulent type strain, nor did the serum from the virulent strain agglutinate the variant. The above statement held good so long as the virulent strain was protected from disintegration, but if the type-specific complex was disrupted, either mechanically by grinding, or by the action of the animal tissues in the process of immunisation, then the virulent strain reacted in agglutinogenic capacity and in agglutinability like the variant. This is an important point—the maintenance of the integrity of the virulent antigen—and it supports the contention that for the purpose of classification by serological reactions the organism must be considered as a whole, in which each and every part of its substance goes to the building up of the molecules of type-specific antigen; that is to say, the serological reactions of the whole intact organism must be used, not one or other or even the sum of the reactions of the various fractions of heat labile and heat stable antigens which may be derived from it.

It should be recognised that the type-specific antigen complex of many bacteria is apt to be broken up before it reaches the antibody-forming cells,

with the result that it is exceedingly difficult to prepare a type-specific serum with them. This difficulty is very marked for example in the case of the Type 3 pneumococcus, and, as it appears to me, it is responsible for at least some of the confusing results in serological work on haemolytic streptococci.

In the Salmonella group variations in type-specificity are represented by the group and specific phases (Andrewes, 1922) which, I suggest, are comparable serologically with the R and S varieties of the pneumococcus. On this hypothesis the group and specific phases of a Salmonella type are the result of the waxing and waning of its functional activity in elaborating type-specific antigen, just as the antigenic differences between R and S pneumococci depend upon the presence or absence of the property of secreting the type-specific carbohydrate. Andrewes has shown that the capacity to agglutinate with a type-specific serum, *i.e.* to produce type-specific substance, is never entirely lost in the group organism, while it is rare for the type-specific organism to be functioning so perfectly that there is none of the group antigen exposed and available for union with the corresponding antibody in a group serum. Similarly, the antigenic distinction between the R and S pneumococci is not always sharp. I have shown on the one hand (1928) that S colonies of pneumococci may after several days' incubation develop foci which fail to react with the type-specific serum, and on the other hand that certain strains of pneumococci growing in the R form may still produce type-specific carbohydrate and exhibit virulence for mice without reversion to the S form. The analogy between pneumococci and Salmonellas is supported by Dawson's (1934) observation that a further stage of degeneration in the R pneumococcus gives rise to the appearance of a variant corresponding more closely than the original R form to the rough form of a Salmonella.

For the following information on bacilli of the dysentery group I am indebted to my colleague, Dr W. M. Scott. In the case of the Flexner dysentery bacilli no complete study of antigenic variation, in the sense of type-specific and group variants, has been published, though there are indications of its existence in the serological studies of Andrewes and Inman. Dr Scott however has made observations, not yet published, on certain of the Flexner types (especially W and Y) which present a close analogy to the group and specific variation which I have noted with *Str. pyogenes*, especially in the tendency to degradation to the group variant on prolonged cultivation (cf. the Hiss and Russell and Y strains of Andrewes and Inman), and in the sharp type differentiation which can be demonstrated by the use of "pure" sera (by heterologous absorption) as compared with "crude." This group degradation of the Flexner bacteria, he considers, is distinct from the "rough" modification, in which both group and type-specific antigen disappear. In the case of the Kruse-Sonne dysentery bacilli degradation to the "rough" form in culture is very rapid, and any intermediate stage is difficult to identify.

One might assume the existence of a basic organism for each bacterial group, viable but without specific pathogenic properties and undifferentiated in consequence of its inability to elaborate type-specific substance. When such an organism is injected into an animal, the result is the formation of an antibody to the ground substance of the bacterium which is common to all

organisms in the group. There is no reason why this ground substance should be split up in exactly the same way in every animal, or indeed why its structure should be identical in every type of the group. If about half the substance of such a basic organism is exercising its function and elaborating the type-specific haptene, injection into an animal will result in a serum with both group and type-specific qualities; in the test-tube on the other hand its antigen will be accessible both to group and specific agglutinins. A fully pathogenic organism, like a highly virulent pneumococcus, with no part of its basic antigen exposed will be quite inaccessible to agglutinins prepared with the avirulent pneumococcus. Similarly, a Salmonella in the type-specific phase may be quite incapable of absorbing group agglutinins.

I have no intention of questioning the importance of the modern method of antigenic analysis in the classification of bacteria. Among the many outstanding discoveries it has accomplished one has only to recall the work of Avery and Heidelberger on pneumococcal carbohydrates, the classification of streptococcal groups by means of precipitins by Hitchcock and by Lancefield, and the classification of the Salmonellas by White. Moreover, group factors supply a basis for interesting speculations on the relationships and evolution of bacterial types. The point which I wish to emphasise is that agglutinating sera for the identification of serological types should be prepared from the fully equipped, actively pathogenic organism, modified as little as possible by treatment after isolation.

I have discussed above a possible analogy between the R and S forms of the pneumococcus on the one hand and the group and type-specific phases among the Salmonellas on the other, and have suggested that the phenomena in the two instances are identical in principle, depending on variations in the activity of the function which has to do with the elaboration of type-specific antigen. It will be of interest now to compare related phenomena in *Str. pyogenes* group and to ascertain how far the above principles are applicable to them.

It has been suspected that many of the anomalies experienced in agglutination and absorption tests with haemolytic streptococci might be explained on the basis of variation in type-specificity. I have examined many colonies, both of the same and different morphology, from different streptococcal types, and have compared their agglutinability towards homologous type-specific sera, *i.e.* sera purified by absorption with heterologous streptococcal types. In only a few instances have marked differences in agglutinability between individual colonies been observed, and these have occurred with types which formed colonies of the watery variety, although there were no morphological differences between the agglutinating and the non-agglutinating colonies. One gained the impression that irregularities in the agglutination reactions of streptococci might be attributable to a gradual loss of the type-specific element and a corresponding degree of exposure of the basal structure, which is the group antigen. Thus a strain which on isolation from a human source was

highly type-specific underwent an alteration in its agglutinability, so that it reacted not only with its purified homologous serum but also with certain heterologous sera. But a complete loss of type-specific antigen, resulting in an inability to react and cause clumping with the homologous agglutinin in a serum from which group agglutinins had been removed, very rarely occurred in the conditions under which the stock cultures have been maintained. A closer study of certain types, however, revealed indications of a phase variation. In the case of Types 3, 13 and 19, the instances referred to above, there was a definite separation into group and type-specific colonies and subcultures of these colonies showed the same fluctuation of phases which has been observed in the Salmonellas. For example, the separation has occurred even in the primary plate cultures from the throat, and on occasions it has happened that the colony selected for test of type has been predominantly group in character. After repeated subcultures in succession, each of which was tested against the diagnostic type sera, the culture has developed type-specific characters, and it has become possible to determine to which type it belonged. A satisfactory explanation of these fluctuations in agglutinability has been found from observations of the growth of these relatively unstable types, particularly Type 3, on a clear agar medium containing a proportion of homologous agglutinating serum. It can be demonstrated that a culture of Type 3 consists of individual organisms, which develop into colonies with varying proportions of type-specific substance. When grown on ordinary serum agar or on horse blood agar there are no differences between the colonies which are type-specific and those which are non-type-specific. But on the homologous serum agar one can see at a glance what the chances would be of obtaining by haphazard selection a type-specific or a group culture. Instead, one can take at once a type-specific colony or even the type-specific portion of a mixed colony. Although at present it is only with Type 3 that the best results have been obtained, it seems probable that this method of cultivation on immune serum agar will be helpful in estimating the effect of various procedures on the type-specific properties of strains, and in determining the possibility of transforming one type of *Str. pyogenes* into another.

The evolution of serological types in nature is an interesting subject for speculation. It seems probable from the severity of scarlatinal epidemics in the past that the *Str. pyogenes* was originally much more virulent and toxigenic, but there is no information available with respect to its serological constitution. In consequence of experience of streptococcal infection being more common in crowded urban communities man has developed a considerable degree of herd immunity, and it may be that the resistance which the streptococcus has had to contend with has resulted in the development of the existing multiplicity of serological races. Each of these races or types differs, however slightly, from another in infectivity, invasiveness and toxigenic capacity in virtue of its individual antigenic constitution. Thus spread of disease in a community means ultimately not only increased resistance on the

part of the host but also alteration and attenuation of the parasite. In this country epidemic scarlatina is mainly due to *Str. pyogenes* Types 1 and 2, but there is considerable evidence, in view of the number of occasions where certain strains of these types have caused tonsillitis outbreaks without rashes, that these types can lose their toxigenicity. Scarlatina is already a very mild complaint and it seems, if the present tendency is maintained, that it will disappear as a definite clinical entity.

VIII. SUMMARY

The haemolytic streptococci associated with scarlet fever, tonsillitis, septic conditions, etc., belong to one group or species designated *Streptococcus pyogenes*.

Cultural and serological aids to the identification of *Str. pyogenes* are described.

The epidemiologically significant types of *Str. pyogenes* appear to be about 20 in number, though probably more than thirty type sera will be necessary for the complete analysis of the group. So far twenty-seven individual serological types have been defined.

The irregularities which have been observed in the agglutination reactions of streptococcal cultures are ascribed chiefly to variations in type-specificity.

Different colonies may give "type-specific" or "group" agglutination, and these distinctions are revealed macroscopically in the case of certain types by growth on homologous agglutinating serum agar.

Some examples are given of observations on the epidemiology of streptococcal infections in schools and other institutions.

The principles involved in the serological classification of a bacterial group are discussed.

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