ON THE IDENTIFICATION OF PNEUMOCOCCI AND THE TESTS EMPLOYED FOR DISTINGUISHING THEM FROM STREPTOCOCCI.

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Fraenkel, in 1886, was the first to isolate and describe a lanceolate diplococcus, afterwards named "Pneumococcus," but to this day the term has lacked definition. It is still too often used rather vaguely to denote all Gram-positive diplococci found in the respiratory tract having pointed outer ends and giving a diffuse growth in broth cultures. Such use of the term is unfortunate since it includes a very large number of organisms which are seldom, if ever, the cause of pneumonia, but which are found with great frequency in the mouth and nose, sometimes certainly associated with catarrhal conditions of the part, but quite frequently in the absence of any such inflammation. This paper attempts to define more closely a group of organisms which, as well as possessing the morphological character of Fraenkel's *Diplococcus pneumoniae*, appear also to be related to the cause of pneumonia.

For some years the possession of a well-defined capsule was regarded as a property peculiar to the "Pneumococcus," but there is now no doubt that capsules are common enough amongst streptococci which could not reasonably be described as pneumococci, and this criterion is therefore fallacious. More specific are the tests of bile-solubility and virulence for mice, both of which together exclude a number of mouth saprophytes from the pneumococcal group. Lanceolate diplococci occurring in faeces, and in faecally contaminated matter, are not likely to be confused with pneumococci, since the frequent presence in faeces of streptococci possessing similar morphology to pneumococci, but with bile-resisting and heat-resisting properties, has for long received very general recognition. But without further careful examination in addition to morphological and cultural study, a large number of the organisms encountered in the respiratory tract and occasionally in distant parts of the body may receive the designation of pneumococcus without any right to the name, and the confusion existing in the literature as to what are and what are not the properties of pneumococci is largely traceable to this cause. How commonly diplococci may be isolated from the pharynx whose shape and colony are indistinguishable from a pneumococcus, but which fail to dissolve in bile, experience in working with such organisms affords ready proof. Unless

these strains are put to further tests the chances are in favour of their inclusion in the pneumococcal group, and on this account it is more than probable that the reported incidence of pneumococci is too high, as even now further tests are not universally applied. For these reasons it has seemed advisable to examine from a critical point of view the alleged properties of pneumococci, and to estimate how far these properties are constant to the species, if indeed the group may be dignified with the name "species."

All Gram-positive diplococci at all resembling the classical "*Diplococcus lanceolatus*" have been considered, with the exception only of organisms occurring in faeces or faecally contaminated matter. The number of strains examined up to the present has not been large, and comprises only sixty-six. The majority were isolated from lesions of the respiratory tract, and the others, with few exceptions, from blood culture. All these strains were regarded as possible pneumococci, and most of them, either by reason of the shape of the cocci or their cultural properties, or both, were at the outset practically indistinguishable from pneumococci. A provisional basis of classification rested on the bile-solubility test, and for the purpose of the investigation the bile-soluble strains were regarded as pneumococci, while the bile-insoluble were designated streptococci.

The question whether bile-solubility affords a criterion for distinction is still an open one. As both Mair (1916), and later Glynn (1923) have pointed out, a certain number of organisms exhibit a partial solubility and are therefore classed as borderline strains, while others are soluble at one time but not at another. Further, some bile-insoluble strains are agglutinated by pneumococcal type sera, as occurred with 2377 and P. 707 (Table IV) both of which were readily agglutinated by Type III pneumococcal serum. It is debatable whether such organisms should be classed as pneumococci or streptococci, but for practical purposes it has been deemed wisest to accept the bile-solubility test as a working method of distinction. The sharpness of the test and the compatibility of its results with results of other tests will be shown below, and it is sufficient here to note that no anomalies arose from this assumption[1].

The bile-insoluble organisms, regarded as streptococci, were subgrouped, on account of their cultural properties and their ability to ferment certain of Gordon's sugars (1905), as *Streptococcus salivarius* (including *S. mitis*), *S. anginosus*, *S. faecalis* and *S. pyogenes*, details of which will be given under the heading of inulin fermentation. The more elaborate classification worked out by Holman (1916) has not been employed because it appears to be just as arbitrary as the earlier system, more complicated, and of questionably greater practical value for distinguishing the various kinds of streptococci.

[1] Since this was written a strain has been isolated possessing all the characteristics of a Type I pneumococcus, with a high virulence for mice and capable of setting up a lobar pneumonia in rabbits, but completely insoluble in bile. Broth cultures, however, show definite autolysis, commencing after 18 hours' incubation.

It is doubtful, for instance, whether haemolytic power, which Holman stresses so heavily in his classification, is so sharply defined or so constant as he would have us believe. It is, however, probable that broad distinctions do exist and are of practical value, and the results of Gordon's sugar fermentation tests over a number of years have seemed to justify their continued employment if they are not interpreted too rigidly and if other characters such as morphology and cultural behaviour are also considered. Short of serological reactions, this method has therefore been deemed the most satisfactory for the present work.

The various properties of pneumococci will now be considered in detail and compared with the properties of those bile-insoluble streptococci which most resemble them on account of their pointed outer ends, their fairly clear colony on solid media and their usually diffuse growth in broth culture. (Other strains of streptococci, not possessing all these characters, have been included for sake of comparison.)

(*a*) *Morphology.*

The shape of the cocci may be no guide in distinguishing the bile-soluble from the insoluble strains. In film, as already noted by Avery (1917), pneumococci may show considerable variation in shape. Round, oval and bacillary forms have not infrequently been seen, and even in films taken from young agar cultures it is often impossible to say whether the organism will prove bile-soluble or not. In pus from empyema thoracis chain formation in pneumococci is common, and without further evidence the diagnosis of streptococcal infection would certainly be made; on the other hand, consideration of the nature of the pus should prevent this mistake since the pleural exudate in streptococcal infection is serous, with flakes of lymph in it, whereas pneumococcal pus is usually thick, creamy and more homogeneous. The results of observing the morphology alone brought the conviction that though few pneumococci are missed, a large number of streptococci are wrongly counted as pneumococci.

(*b*) *Cultural characters.*

Very considerable similarity was found between the colonies of pneumococci and certain strains of streptococci, when grown on a specially prepared agar medium—Gaskell (1924)—containing heated blood extract. On this medium both pneumococci and streptococci grew well, the colonies of pneumococci, if well spaced, showing a diameter of 3 mm. or more after 18 hours' incubation. The more delicate and clearer colonies were considered in detail, and a large proportion of them proved to be bile-insoluble. It was usually impossible to state with any certainty whether a colony of 18 hours' growth was pneumococcus or streptococcus. The clarity, consistency, shape and size were considered in turn, and the results in tabular form offered no reliable means of distinguishing the pneumococci from the others. Some strains of both species produced colonies with a central depression, while other strains

gave colonies with a domed centre and a raised circumference. Occasionally neither of these shapes occurred, and the colony presented a fairly flat surface. Longer cultivation than 18 hours, however, brought out in most cases features which enabled the species to be distinguished in some degree from one another. Three tendencies in pneumococcal, that is bile-soluble, strains were particularly noted; their viability compared with streptococci was low—they usually died after two or three subcultures on ordinary media—whereas streptococci grew well; autolysis commenced early and showed itself by a progressive thinning of the colony, starting in the centre and spreading outwards; and the formation of secondary smaller colonies at the periphery was more frequent in, but not invariably confined to, pneumococcal strains.

Growth in a specially prepared broth medium, Gaskell (1924), proceeded after 18 hours' incubation to a concentration of about 500 million cocci per c.c. The bile-insoluble strains that grew diffusely in this broth, tended to grow more rapidly than the pneumococci, and continued growing for 2, 3 or 4 days giving as a rule a very dense turbidity, whereas the pneumococci showed a maximum concentration in 18 hours, after which thinning took place by progressive autolysis until only a faint turbidity persisted. This thinning was not observed in any of the streptococcal cultures, and therefore offers a fairly reliable criterion for distinction. As regards chain formation in broth there appears to be little difference between the diffusely growing streptococci and the pneumococci. In either case chains of cocci up to twelve in number are not uncommon, while the pointed shape and diplococcal arrangement as a rule remain unaltered in both species.

(c) *Capsule formation.*

As mentioned above, importance was originally attached to capsule formation for distinguishing pneumococci from other similar diplococci. It is true that *all* the bile-soluble strains examined had readily demonstrable capsules, *vide* Table II, but examination of several strains of streptococci, both *salivarius* and *pyogenes* types revealed capsules too, *vide* Table III, in which the six strains considered at random all showed capsules. Encapsulation therefore affords no criterion for distinction.

Capsules were best exhibited in the blood films taken from a mouse dying of septicaemia from the intraperitoneal injection of the strain under consideration. Avirulent strains often showed good capsules when grown in Hiss's serum water. The following modification of Hiss's method of capsule staining was found the most satisfactory.

1. Stain by heating the *unfixed* film in 5 per cent. alcoholic solution of fuchsin in distilled water, for one minute.

2. Wash off with 20 per cent. copper-sulphate solution, and blot dry.

Longer exposure to the decolourising action of the copper sulphate is apt to remove all stain from the capsules, which should show as mauve halos around the diplococci, with sharply defined outer margins.

In accordance with expectations, the best capsules were shown by freshly isolated strains; in those kept long in vitro, such as the National Type Pneumococci, good capsules were not demonstrable. Capsules are probably products of metabolic activity in the growing cocci, whether pneumococci or streptococci, and vary in a particular strain with the metabolic rate so that the more slowly growing cultures may often show only ill-defined capsules or even none at all.

(*d*) *Inulin fermentation.*

Inulin fermentation has been, and still is, generally regarded as a property highly characteristic of pneumococci, if not peculiar to them. It is so regarded by the following authors in their standard text-books on bacteriology, viz. Muir and Ritchie (1927); Hewlett (1926); and Hiss-Zinsser (1926), etc. Avery and others (1917) were so sure of this that they remark that the test is of differential value in distinguishing pneumococci from streptococci, though they admit that some strains fail to ferment this sugar, while others do so but inconstantly. Andrewes and Horder (1906) using Gordon's Lemcopeptone medium, found only 25 per cent. of their pneumococci fermented inulin, but they note that the results are vitiated by the fact that pneumococci did not grow well in their medium. Also it is doubtful whether a large proportion were true pneumococci since the bile-solubility test was not then in vogue. The American statistics, on account of this added means of distinguishing pneumococci, should show a nearer approach to the truth, but my own results do not agree with the American observations, for not only do some strains of pneumococci fail to ferment inulin, but a large percentage of streptococci of the salivary type possess this power to a high degree.

Glynn (1923) has also come to the conclusion that inulin fermentation occurs with a considerable proportion of streptococci, and attributes much of the disagreement on the subject to the different media and the different techniques employed in the test. Before discussing results in detail therefore it will be well to consider some of the difficulties to be overcome in the performance of the test. The amount of acid formed even in a rapidly growing culture is small and the following conditions must be observed if error is to be avoided. A good pabulum is essential for such delicate bacteria as pneumococci and streptococci; the reaction of the medium most favourable is about *p*H 7·8; there should be a low concentration of buffer-salt, so that the changes in reaction shall be considerable when small amounts of acid are formed, while a sensitive indicator, such as phenol-red, is necessary for registering these changes; lastly, the medium should contain no fermentable substance which may easily give rise to the diagnosis of false positives. Unless due regard is paid to all these points, it is likely that misleading results will be obtained. Positives will be missed owing to poor growth or an insensitive medium, and other positives will be wrongly given in cases where the fermentation is not due to splitting of the inulin.

As a medium Hiss's inulin-serum-water is widely recommended in text-

books, monographs, etc., and in most respects it is excellent. But one fact is apt to be lost sight of in employing it, namely that it contains a fermentable substance in quantity sufficient to interfere with the test, and only by carefully controlling each fermentation reaction can the possibility of gross error be eliminated. The substance, which gives the reactions of glucose, is derived from the bullock-serum, which, however freshly obtained from the animal, always contains sugar—in small amount it is true but sufficient to vitiate the test. This fact is not mentioned by the American observers, nor have I seen it mentioned elsewhere, so that therein lies a possible source of error accounting for some of the discrepancies in reports on the subject. In the present series the results, given in Table I, of investigating this point in the case of six strains of pneumococci, nine strains of *Strept. salivarius*, and six of other streptococci, leave no doubt as to the presence of fermentable sugar in Hiss's medium. It will be noted that fermentation of the medium occurred to a marked degree—even to coagulation in one case—with no less than eighteen of the twenty-one strains, whereas fermentation of the inulin, recorded after allowance for fermentation of the serum, occurred with only fourteen of these strains. Thus at least seven false positives might have been given. It is therefore essential in using Hiss's medium to put up control tubes of the medium without inulin, in order to allow for the considerable fermentation of the contained glucose which takes place in nearly every case.

Table I. *Showing fermentation of Hiss's serum-water medium owing to traces of glucose.*

Refer. no.	Organism	Fermentation of serum-water alone	Fermentation of serum-water + inulin
2579	Pneumococcus	O	AC
E. 13	,,	A	AC
2744	,,	A	AC
P. 787	,,	A	AC
2783	,,	A	AC
P. 788	,,	A	AC
P. 742	*Streptococcus salivarius*	A	AC
2596	,,	A	AC
2377	,,	Tr	AC
P. 707	,,	O	AC
2738	,,	A	AC
P. 758a	,,	A	A
P. 758c	,,	A	AC
P. 794	,,	A	AC
P. 777a	,,	Tr	AC
P. 758b	*Streptococcus anginosus*	A	A
P. 758d	,,	AC	AC
P. 777b	*Streptococcus pyogenes*	A	A
S.	,,	A	A
2743	,,	A	A
2764	,,	A	A

Indicator—phenol red.

O = no recognisable fermentation (*p*H 7·8 approx.).
Tr = trace only.
A = moderate fermentation (*p*H 6·6 approx.) no clotting.
AC = marked fermentation, with clotting of medium (about *p*H 6·0).

The desirability of a sugar-free medium has sometimes been stressed, and theoretically such a medium has obvious advantages. In practice difficulty is encountered in the preparation of a sugar-free broth which will retain its growth-supporting characters, for a really good nutrient broth when deprived of its sugar in the usual way—by growing *B. coli* in it, filtering and correcting acidity—may so far lose its growth-supporting properties that only with great difficulty can pneumococci or streptococci be induced to grow in it, growth being so feeble that fermentation is hardly detectable. The only organism growing at all satisfactorily in such a broth was a *Strept. faecalis* which is well-known as a hardier and less fastidious type than most of the streptococci. It is true that organisms grew more abundantly in the sugar-broths which were most markedly fermented by them, as in the inulin broth with certain strains of pneumococci and in the mannite broth with the *S. faecalis*, but with the exception of the latter the multiplication of organisms in this medium was so slight as to be hardly recognisable. It was further noted that the reaction of the "sugar-free" broth was changed somewhat towards the acid side by all the strains examined, so that a trace of fermentable substance remained even after the *B. coli* had been grown in it for twenty-four hours, while longer than this allowed the reaction of the medium to reverse, probably owing to some proteolytic action of the *B. coli*.

The advantages of using a sugar-free medium are therefore considerably fewer in practice than in theory, and probably the most satisfactory method of testing the fermentative powers of these organisms is by growing them in Hiss's serum-water, or in a good nutrient broth prepared from fresh bullock's heart. Both of these media admittedly contain small quantities of glucose, but by putting up control fermentation tubes of the serum-water, or the broth, alone with the organism, any acid changes due to glucose fermentation can be duly allowed for in each case.

The result of testing out the inulin-fermentative powers of forty-five strains of pneumococci are given in Table II, and of forty-one streptococcal strains of various types in Table III. Either Hiss's medium or a good broth medium, Gaskell (1924), was used, and in every case allowance was made, by inoculating a control tube of the medium, for fermentation of contained substances other than the sugar actually tested. Seven days was found to be long enough to allow for the slowest inulin fermenters, but forty-eight hours was usually ample time.

The bile-insoluble cocci have been divided on account of their growth in broth and their behaviour when grown in mannite and litmus milk, into three groups, *Streptococcus salivarius*, *S. anginosus*, and *S. pyogenes* (Gordon's classification). Those forming a deposit in broth with clear supernatant fluid have been regarded as *S. pyogenes* unless they caused clotting of litmus milk in one week, in which case they were classified as *S. anginosus*; the latter characteristically gave a *mucoid* deposit, while the *S. pyogenes* gave a more scanty and *granular* sediment. Those bile-insoluble organisms showing little or no

Table II. *Showing fermentative and other characteristics of pneumococci.*

All strains gave a diffuse growth in broth and were bile-soluble.

Refer. no.	"Type"	Capsules	Inulin fermentation	Virulence titre
P. 468	I	+	+	7
E. 13	I	+	+	6+
2219	I	+	0	6
2579	I	+	+	6−
2744	I	+	+	6−
P. 805	I	+	0	6−
P. 787	I	+	0	5+
P. 788	I	+	+	5+
83.26	I	+	0	5+
3523	I	+	+	5+
2611	I	+	+	5
2811	I	+	+	5
2374	I	+	+	4+
3482	I	+	+	4+
2783	I	+	+	4+
3089	I	+	0	4
3362	I	+	+	4
2356	I	+	+	4
2305	I	+	+	4−
2210	I	+	−	3+
2409	I	+	+	3+
2311	I	+	+	3
3453	III	+	+	5+
2043	III	−	+	4+
2174	III	+	+	3+
2229	III	+	+	3
2461	III	+	+	3?
3260	III	+	0	3−?
E. 27	IV	+	+	5+
24.26	IV	+	+	5−
P. 943	IV	+	0	5−
3291	IV	+	0	5−
46.26	IV	+	0	3+
P. 826	IV	+	+	3
P. 820	IV	+	+	2
2906	IV	+	0	1+
13.27	IV	+	+	1+
P. 1003	IV	+	0	1−
1876	IV	−	0	1
P. 470	IV	+	0	0
P. 566	IV	+	+	0
2812	IV	−	0	1?
P. 699	IV	+	+	0
2914	IV	−	+	0
2407	IV	+	+	0

+ = present. 0 = absent. − = not examined.

Virulence titre = the logarithm of the reciprocal of the M.L.D. in c.c. of 18-hour broth culture, *e.g.* if M.L.D. is 1/10 c.c. the virulence titre is *1*, and if M.L.D. is $1/10^x$ c.c. the virulence titre is x.

Total number of strains	45
Inulin fermenters	30
Percentage of inulin fermenters	67

deposit in broth, giving a diffuse turbidity which failed to subside after several days, and lacking the power to ferment mannite were regarded as *S. salivarius*. That these broad distinctions have some significance is likely, since all save two of the *salivarius* and *anginosus* strains came from the respiratory tract, chiefly the mouth, nose and pharynx, while eight of the twelve *S. pyogenes* were associated in pure culture with definite suppuration. The *S. salivarius*

strains all showed strong resemblances to pneumococci, both in their morphology and in broth and agar culture, and it was usually impossible to be certain whether the organisms were not pneumococci until the virulence and bile-solubility had been tested.

The most interesting fact in connection with the *salivarius* strains is that fifteen of the twenty-one quite definitely ferment inulin. In every case the

Table III. *Showing fermentative and other characters of streptococci.*

Refer. no.	Organism	Broth culture	Bile-solubility	Capsules	Fermentation: Inulin	Fermentation: Mannite	Fermentation: Litmus milk
P. 742	*Streptococcus salivarius*	T	0	+	+	0	AC
2377	,,	T	0	+	+	0	A
2596	,,	T	0	+	+	0	A
P. 707	,,	T	0	+	+	0	AC
2738	,,	T	0	–	+	0	AC
P. 794	,,	T	0	–	+	0	AC
P. 777a	,,	T	0	–	+	0	AC
P. 758a	,,	T	0	–	0	0	A
P. 758c	,,	T	0	–	+	0	AC
2847	,,	T	0	–	0	0	A
P. 869a	,,	T	0	–	0	0	AC
P. 871	,,	T	0	–	+	0	AC
P. 1206b	,,	T	0	–	+	0	AC
P. 1062	,,	T	0	–	+	0	AC
17.27	,,	T	0	–	+	0	AC
P. 1084	,,	T	0	–	0	0	AC
3604	,,	T	0	–	0	0	AC
P. 1103a	,,	T	0	–	+	0	AC
P. 1162	,,	T	0	–	+	0	AC
P. 1206a	,,	T	0	–	+	0	A
27.27	,,	T	0	–	0	0	AC
P. 758b	*Streptococcus anginosus*	D	–	–	0	0	AC
P. 758d	,,	D	–	–	0	0	AC
2798	,,	D	–	–	0	0	AC
P. 869b	,,	D	–	–	0	0	AC
3756	,,	D	–	–	0	0	AC
P. 1194	,,	D	–	–	0	0	AC
P. 1054	,,	D	–	–	+	0	AC
P. 1103b	*Streptococcus pyogenes*	D	–	–	0	0	A
3883	,,	D	–	–	+	0	A
P. 739	,,	D	–	+	0	0	A
S.	,,	D	–	+	0	0	A
P. 777b	,,	D	–	–	0	0	A
2743	,,	D	–	–	0	0	A
2764	,,	D	–	–	0	0	A
2992	,,	D	–	–	0	0	A
2881	,,	D	–	–	0	0	A
P. 879	,,	D	–	–	0	0	A
2603	,,	D	–	–	0	0	A
P. 751	,,	D	–	–	0	–	A
2669	,,	D	–	–	0	–	A

D = deposit only, with clear supernatant broth.
A = acid formation.
T = turbid growth.
0 = absent.
C = clotting (complete).
+ = present.
– = not examined.

Streptococcus salivarius.

Total number of strains	21
Inulin fermenters	15
Percentage of inulin fermenters	71

(N.B. Only single colony subcultures were employed, so that mixing of strains was as far as possible excluded.)

cultures used for fermentation tests were made from single colony subcultures, so that there would be no likelihood of including a strain of pneumococcus amongst the streptococci, or *vice versa*. It appears therefore that about 71 per cent. of bile-insoluble *S. salivarius* strains taken at random are capable of fermenting inulin. The figure compares favourably with those for the pneumococci examined, of which only 67 per cent. are inulin fermenters.

This high incidence of inulin fermenters amongst *S. salivarius*, contrasting with the almost complete absence of inulin fermentation amongst the other types of streptococci—P. 1054 and 3883 being the sole exceptions—helps to bring this group, already closely related, still closer to the pneumococci. For the same reason the inulin fermentation test in affording such similarities can have not the slightest differential value for distinguishing these two closely-allied species, though it does further mark off *S. salivarius* from the other types of streptococci.

(*e*) *Virulence for mice.*

Pneumococci are generally known to possess a high order of virulence for mice when injected into the peritoneum, but pathologists are often content to pay no attention to the dosage so that doses of various size are given in different cases and by different workers, and the conclusions reached are therefore in no way comparable. For instance, whereas 1 c.c. of a broth culture of a given strain may kill a mouse within 24 hours, 0·1 c.c. may produce no recognisable effect at all. With the larger dose which causes death the strain would be regarded as virulent, while if only 0·1 c.c. were injected the result would seem to point to absence of virulence for mice. It has been shown repeatedly that virulence varies in degree with pneumococci and streptococci as with other organisms, and for any useful conclusions to be drawn the minimal lethal dose of the organism must be found. The principle has been applied in America for some time, and is becoming more general in England in routine practice. In a recently published paper Gaskell (1924) has defined the virulence of a strain in terms of the minimal lethal dose of an 18-hour broth culture, suitably diluted if necessary ten times, a hundred times, a thousand times, etc., in normal saline. The density of such a culture is remarkably constant, only varying between 200 million and 500 million cocci per c.c., a small variation in comparison with the magnitude of the dilutions used. By using only 15–20 gram mice and allowing no time to elapse between dilution of the culture and injection, approximately standard conditions can be attained. By applying this method to streptococcal as well as pneumococcal cultures it has been found possible to compare the virulence of different strains of pneumococci with various strains of streptococci. For convenience the results have been expressed as integers obtained by taking the logarithm of the reciprocal of the minimal lethal dose in c.c. of a broth culture. In more ordinary language, if the lethal dose is 1/10 c.c. the virulence is 1, while if the M.L.D. is 1/100,000 c.c. the virulence is 5, and the greater the virulence the

larger is the integer, so that this figure affords in some degree a quantitative measure of virulence.

The method has been shown in another paper, Whittle (1927), to be applicable to any strain, no matter in what exudate it occurs nor how mixed with other organisms, and a quantitative means of comparing the virulence of strains taken from all kinds of exudates has thus been established. The forty-five strains of pneumococci were tested for virulence in this way and the results given in Table II, last column; and the virulence of twenty different strains of freshly isolated streptococci taken at random is given in Table IV.

Table IV. *Showing virulence of streptococci.*

Refer. no.	Type	Lesion	Course of disease	Virulence titre
35.25	*Streptococcus pyogenes*	Septicaemia	Death	2
2343	,,	,,	,,	1 –
2134	,,	Erysipelas	Recovery	1
P. 474	,,	Abscess	,,	1
2357	,,	Pleurisy	,,	1 –
P. 707	*Streptococcus salivarius*	Chronic bronchitis	,,	1 –
2377	,,	Endocarditis	Death	0
42.24	*Streptococcus pyogenes*	Pericarditis	—	0
P. 440	,,	Pleurisy	—	0
P. 508	,,	,,	—	0
P. 392	,,	Meningitis	—	0
1622	,,	Septicaemia (puerperal)	Recovery	0
P. 712	*Streptococcus longus*	Nasal catarrh	—	0
P. 434	,,	Pharyngitis	—	0
1539	,,	Influenzal pneumonia	Death	0
2217	*Streptococcus brevis*	Chronic bronchitis	—	0
P. 486	,,	Scarlet fever urine	—	0
P. 493	,,	Carious tooth	—	0
1826	,,	Abscess	—	0
1815	,,	Angina, tonsil	—	0

For "Virulence titre" see Table II.

Note. The strains included in this table were obtained, in most cases, in pure, or practically pure, primary culture. Those not isolated in pure culture occurred in such numerical predominance as to suggest their being the most important causative organism present.

It will be noted at a glance that there is wide variation in virulence between the pneumococcal strains, some being as much as 10^7 times as virulent as others, but that 75 per cent. of the pneumococci show a high order, titre 3 or more, while only six of the twenty streptococci possess a detectable virulence for mice and this of a comparatively low order, maximum *2* in contrast with the pneumococci maximum *7*.

Table V. *Comparison of virulence of pneumococci and streptococci for mice.*

Organism	Maximum virulence	Minimum virulence	Highly virulent (titre 3 or over)	Slightly virulent or avirulent (under titre 3)
Pneumococci	Titre 7	Titre 0	75 %	25 %
Streptococci	Titre 2	Titre 0	0	100 %

The results, summarised in Table V, show that pneumococci occurring in human lesions are as a rule highly virulent to mice, but that streptococci, even from lesions which prove fatal, only exceptionally possess this power, and then in comparatively minor degree.

Gaskell (1925) has shown that previous failures in attempts to produce experimental lesions in rabbits by intra-bronchial insufflation of pneumococci are attributable to a subminimal virulence in the strain employed. It is evident from observations not yet published that high virulence, such as may be gauged by the effect on mice, is a factor of fundamental importance in determining the type of pulmonary lesion in the human subject. A later paper will deal with the subject in greater detail and it will be sufficient to remark here that the high virulence characteristic of the majority of pneumococci is absent in all the types of streptococci, even in those recovered from fatal lesions.

(f) Bile solubility.

The solubility of pneumococci in bile was accidentally discovered by Neufeld in 1900 while working on immunity. It took some years for the discovery to be applied to routine practice in distinguishing pneumococci from other organisms, and no doubt the variation in solvent power of different samples of bile did not hasten its adoption. Whole bile was not used in the present investigation because in preliminary trials it gave a cloudiness in broth cultures which completely masked any solvent action it may have exerted. Instead sodium desoxycholate was used, a salt recommended by Mair (1917) on account of its solvent power, which he found to be ten times as great as sodium taurocholate or whole bile. It forms a perfectly clear, colourless solution in distilled water and causes no cloudiness on addition to a broth culture, provided the reaction of the broth is correctly adjusted. A 10 per cent. solution was made up for stock and 0·1 c.c. added to 5 c.c. of a broth culture was sufficient to cause complete clearing within three minutes if the strain were bile-soluble. The salt is therefore effective in concentrations of 0·2 per cent. Some difficulty was experienced in procuring a pure salt, one commercial sample failing to give a clear solution in distilled water and another causing cloudiness in broth. Mair (1917) advises purification by recrystallisation and washing with glacial acetic acid, and this method has been successfully employed. The sample actually used in the present work was one very kindly provided by Dr Mair himself from the National Institute of Medical Research, Hampstead.

Certain points in the technique are worthy of mention.

1. The reaction was in every case quite definite, and complete clearing of the broth was accomplished within three minutes or not at all.

2. Too acid a medium causes precipitation of desoxycholic acid which masks the dissolution. The precipitation commences on the acid side of *p*H 6·6, and it may be necessary therefore to correct the reaction of the medium, before adding the bile salt, by the addition of a drop or two of *N*/10 NaOH solution. The test cannot be effected in a broth much more acid than *p*H 6·6.

3. On the other hand, greater alkalinity than *p*H 7·8 tends to precipitate the phosphates in the broth. This too will mask any solution of organisms taking place.

4. Exposure of the cocci to a temperature of 55° C. or higher for ten minutes or so completely prevents subsequent solution by the salt. Suspensions of cocci which have been killed by heating for a few minutes in a water-bath may be used for agglutination tests but are useless for bile-solubility.

Bile-solubility is a phenomenon exhibited by the living organism only, and there is now strong evidence favouring the view that the process is merely a greatly accelerated autolysis which occurs spontaneously under favourable conditions, but taking days instead of minutes as it does in the presence of bile salt. The spontaneous lysis occurring in broth cultures and mentioned above as a means of distinguishing pneumococci from streptococci, has been shown by Atkin (1926) to run parallel with solubility in bile, and there seems little doubt that the bile salt acts by lowering the surface tension thus accelerating a process already taking place spontaneously.

Summary and Conclusions.

The difficulties encountered in distinguishing pneumococci from streptococci, particularly *S. salivarius*, have been enumerated and the morphological and cultural similarities between the two groups of organisms have been pointed out. If bile-solubility is taken as the distinguishing mark of a pneumococcus it will be found that there are a large number of bile-insoluble streptococci commonly met with in the respiratory tract and sometimes playing an important part in disease, which bear very strong resemblances to pneumococci both in shape, colony, and broth culture. The similarities are specially confusing in mixed cultures on agar plates and a number of the routine tests applied quite failed to distinguish them. There naturally follows the corollary that some of these tests fall wide of the mark, and they should therefore be left out of the bacteriologist's armamentarium when the identification of pneumococci is under consideration. Morphological and cultural properties are insufficient guides to species, while the same is true of the widely recommended tests of inulin fermentation and capsule formation. Not only do some pneumococci fail to ferment inulin, but so many strains of *S. salivarius* show active inulin fermentation that, in the present series of organisms taken at random, the incidence of inulin fermenters amongst *S. salivarius* is actually higher than amongst pneumococci. Capsules were found in all the strains of streptococci in which they were sought and in all the pneumococci investigated, so that encapsulation may be regarded as a property common to members of both species.

It may well be asked whether there is any property specific to the pneumococcus other than bile-solubility. A cautious affirmative may be given by recalling the fact that great virulence for mice was shown to be possessed only by pneumococci, and though a small proportion of pneumococci lacked this power, even the most pathogenic streptococci failed to approach in virulence the majority of pneumococci. This lethal power for mice is probably the only property besides a propensity to rapid autolysis associated with bile-solubility,

by which the pneumococcal group can be defined. Its importance, from the standpoint of human pathology, will be discussed it is hoped in a later paper.

It is probable that in the pneumococcus we are dealing with a fairly sharply defined group which it is justifiable to dignify with the name "species." Its shape, cultural characters, bile-solubility and high virulence to mice make up a fairly clear picture. On the other hand, as a species it merges somewhat into the nearly allied group of *S. salivarius*, which though bile-insoluble is similar in many ways to the avirulent strains of pneumococci.

Amongst the streptococci Gordon's pioneer work (1905), elaborated by Andrewes and Horder (1906), has gone far in bringing order to a rather chaotic subject, and some meaning can now be attached to the terms *Streptococcus salivarius*, *anginosus*, *faecalis* and *pyogenes*; though these groupings can hardly claim the right to the term "species." In considering them it may be useful to recall the suggestion put forward by Andrewes (1906), namely, that the streptococcus frequently falls short of true parasitism and that it is only partially stabilised. The various strains encountered represent various stages in its evolution from the harmless saprophyte to the highly virulent parasite met with in fulminating septicaemia and the like.

What is true of streptococci is also partly true of pneumococci. The closeness of the relationship between the less virulent pneumococci and the salivary streptococci shows how the two groups tend to merge at their edges. Yet in spite of this the pneumococcus appears to have evolved a stage further than the rest of the streptococcus group. It is in the main typical, highly specific, and unlike the rest of the streptococci.

The conclusions reached may be summarised thus:

1. Pneumococci constitute a group which can be differentiated from the streptococci, though forms intermediate between the two do most probably exist.

2. The salivary streptococci are very closely related to the pneumococci, and are frequently indistinguishable from them by ordinary means.

3. The chief means of distinguishing them are by rapid autolysis in culture and bile-solubility, and by a very high virulence for mice, these properties being characteristic of pneumococci and absent in streptococci.

4. Inulin fermentation is as common amongst streptococci salivarii as amongst pneumococci, and the power to form capsules is also common to members of both species. As means of differentiation both tests are useless.

5. Hiss's serum-water contains glucose in sufficient quantity to give rise to acid formation when used for fermentation tests, and cannot therefore be used for inulin fermentation unless the acid formed by the glucose fermentation has been allowed for with each strain tested.

The latter part of the work embodied in this paper was completed and the paper written during my tenure of the Ernest Hart Memorial Scholarship of the British Medical Association.

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