THE RELATION OF VIRULENCE IN PNEUMOCOCCI TO DISEASE, WITH A COMPARISON OF VIRULENCE OF THE DIFFERENT TYPES OF PNEUMOCOCCI IN VARIOUS PATHOLOGICAL CONDITIONS.

By C. H. WHITTLE, M.D., M.R.C.P.

Ernest Hart Memorial Scholar of the British Medical Association; Clinical Pathologist, Addenbrooke's Hospital, Cambridge.

(*From the Bonnett Laboratory, Addenbrooke's Hospital, and the Pharmacological Laboratory, Cambridge.*)

Pneumococci form a group of organisms of which the individual strains exhibit wide differences in character. Like the streptococci, certain types of which they closely resemble, they show a variability in their predilections and behaviour bewildering to those who are attempting to classify them. Not only are pneumococci known to cause a great variety of lesions about the body, but they have also been found in the throats of a large proportion of healthy persons who apparently suffer no inconvenience from their presence —Stillman (1916, 1917), Glynn (1923) and others.

Several questions must be answered before these facts can reasonably be reconciled. Is it the same organism that occurs in pneumonia, in meningitis, in abscesses in different parts of the body, and in the normal pharynx, or are there in reality many different organisms alike in shape but differing intrinsically one from another? If, as seems probable, there are many different organisms, what is the nature of their differences, and can these be measured? Lastly, in what degree are such differences stable and constant? Answers to questions of this kind should shed light on the incidence of infection and its mode of spread.

The fact that strains of pneumococci differ in pathogenicity has long been recognised, and, when immunity reactions were introduced for the "typing" of pneumococci fifteen years ago, there was evidently some hope that the respective "types" would represent organisms similar, if not identical, in all important respects, including pathogenicity. That the "fixed types" do include a large number of highly pathogenic strains is true, but a proportion by no means negligible of the inagglutinable "Group IV" strains are far from being harmless saprophytes, and some are admittedly just as highly pathogenic as the "fixed types." Moreover the discovery of other "fixed types" by other workers, *e.g.* Lister (1913), introduced further complications, and the multiplication of "types" and "subtypes," Armstrong (1922) *et al.*, has proceeded to a point where the typing classification becomes unwieldy and apparently indeterminate. For these reasons little confidence can be felt

in typing as a means of ascertaining the relative pathogenicity of strains, a conclusion borne out by figures cited below.

As an index of pathogenicity use has often been made of the virulence of pneumococci for mice, measured in terms of the M.L.D. causing death in 48 hours of a mouse of standard weight and breed. But there has been no systematic attempt along these lines to compare the virulence of different strains. The comparison of virulence of a great variety of strains by this means, however, has been found possible (Whittle, 1928), since the one or two subcultures required to obtain the strain in pure culture do not affect the virulence appreciably (Whittle, 1927). Objection has been raised to the method on the score that only two mice—the *minimum* employed—were inoculated for each dose, and that conclusions drawn from such limited data are untrustworthy, but the sharp end-point obtained in nearly all cases and the constancy of the results tend to do away with such an objection. The figures will probably be found to give a more adequate index of pathogenicity than the typing classification alone can afford.

In this paper the question whether the virulence of a given strain fluctuates has not been dealt with *in extenso*. From the evidence the contrary seems true. But wide differences in virulence between strains recovered from different lesions were noted from the outset, and there was some ground for believing that these differences would be susceptible of orderly arrangement and would bear some relation to the nature and severity of the lesion. The belief arose from the consideration of work on experimental pneumonia in rabbits by Gaskell (1925), which brought to light a singularly constant relationship between the virulence of the infecting pneumococcus and the nature of the lesion in the rabbit. The object of the work described here is to establish a similar relationship between organism and lesion in man.

All strains of bile-soluble diplococci with the characteristic shape, etc., of pneumococci recovered during three years' routine practice have been utilised, together with a few of the many bile-insoluble organisms which at first sight are indistinguishable from pneumococci on account of their shape, cultural characters, fermentative and even immunity reactions, and which are probably closely allied to the pneumococci (Whittle, 1928). Most of the strains were isolated in practically pure primary culture, and those that were not showed respectively such a predominance over other organisms present that theirs was probably the chief rôle in the infection. For the typing classification the three American sera, "Types I, II and III," have been employed, and Lister's "Type A" serum has also been tried on all the strains recovered.

Technique of Typing.

The Type I, Type II and Lister "A" sera were obtained from Dr R. R. Armstrong, who had prepared them by inoculating rabbits with the corresponding cocci. The Type III serum was kindly provided by Prof. Simon Flexner.

The method of agglutination was the one used by Armstrong (1921). For suspensions of cocci 18-hour broth cultures, previously killed by 20 minutes' immersion in the water bath at 55° C., were used. The ultimate dilutions of serum were 1/12, 1/24, 1/48, 1/96 and 1/192. The reaction was seldom complete until 24 to 48 hours after 2 hours' incubation, and the tubes were allowed to stand at room temperature for this period as routine practice. Readings were taken after incubation, after 24 and again after 48 hours. Most of the strains were tested out to dilutions of 1/192, but some gave marked specific reactions in the lower dilutions and were not examined in the higher titres. Control tubes of the organisms without serum were put up in every case.

All strains save one gave specific reactions only. In case any of the inagglutinable Group IV strains might be capable of absorbing one or another of the type agglutinins, and so really belong to one of the "fixed" types, the power of absorption of agglutinin was tried in a few cases with Armstrong's technique—Armstrong (1922). The results gave no further evidence of specific reaction in three virulent and two avirulent inagglutinable strains, even after repeated trials with fresh samples of cocci. For the rest, with few exceptions the agglutination reactions were deemed sufficient.

Table I. *Showing cross-immunity reactions of the National Type Cocci.*

	Type I coccus	Type II coccus	Type III coccus
Type I serum, agglutination	1/96 +	0	0
„ „ absorption	Complete	—	—
Type II serum, agglutination	0	1/192 + +	1/12 +
Type III serum, agglutination	0	1/48 +	1/192 + +
Type "A" serum, agglutination	0	1/12 +	0

Agglutination was tested between the final dilutions of 1/12 and 1/192 of the Type sera.

\+ = visible agglutination.
\+ + = marked agglutination, with tough adherent deposit.
\- = not examined.

In order to see whether the agglutinating properties of the sera were active, tests were made on suspensions of the National Type cocci, cultures of which were provided by Dr St John Brook from the National Institute of Type Cultures. The results are set forth in Table I. These cocci reacted to their proper sera in fairly high dilution, but with the exception of Type I coccus they also showed considerable cross-agglutination with the other type sera. For instance, II coccus was agglutinated by III serum and A serum in reasonably high titre, and III coccus reacted with II serum. Since these cocci grew poorly and were completely avirulent to mice, their behaviour with the type sera may well have been due to their condition of attenuation, rather than to any abnormality in the sera. Other workers, notably Griffith (1923) and Reimann (1925), have observed similar tendencies with various types of pneumococci which, having lost their virulence and other characters proper to them, show marked cross-agglutination with the other type sera, in addition to a specific reaction to their own type serum. Indeed, Griffith (1928) now

claims to have converted one type of pneumococcus into another at will. Findings such as these detract from rather than add to the practical utility of the typing classification. Nevertheless in the present series the majority of freshly isolated strains that were at all agglutinable gave specific reactions to a fairly high titre, and only one showed any tendency to cross-agglutination. Indiscriminate agglutination, such as Armstrong (1923) noted with a few strains, was not encountered, and only one strain showed auto-agglutination.

The results of typing, summarised in Table II, indicate that Type I and Group IV predominate (last column). They contain about the same number of strains and together embrace four-fifths of all the strains. The absence of

Table II. *Comparing the Virulence of the different Types of Pneumococci.*

Type	Highly virulent (titre 3 or more) %	Feebly virulent or avirulent (less than titre 3) %	Average virulence titre	Total number of strains	Relative proportion of different types %
Type I	100	0	5	26	44
Type II	—	—	—	0	0
Type III	88	12	4	9	15
Type "A" (Lister)	1 strain	1 strain	—	2	3
Group IV	39	61	2	23	38
			Total	60	

For "Virulence Titre" see footnote to Table III.

The agglutination tests were carried out with final dilutions of serum ranging from 1/12 to 1/192; a few strains were not tested out in high dilutions.

Type II strains, in Cambridge, 1924–27, is probably fortuitous and corresponds to a low percentage of Type II strains obtained from lobar pneumonia cases by Griffith (1928) in London during the same period. Other workers in other times and places have reported the predominance of different types; *e.g.* Armstrong (1923), Glynn (1923). All the Type III strains gave slimy, confluent growth on solid media, particularly noticeable on egg, but it is doubtful whether this kind of growth is peculiar to Type III since four Type I strains showed the same characters.

VIRULENCE IN RELATION TO TYPE OF PNEUMOCOCCUS.

Virulence is a property not confined to the "fixed" types. For instance, Group IV contains five strains of virulence titre 5 and three of titre 3, that is to say, 8 strains out of 23 in this group are highly virulent. It is wrong, therefore, to regard Types I, II and III as the virulent strains and Group IV the avirulent. Nevertheless, the Type I strains without exception are highly virulent, titre 3–7, and neither Type III (average titre 4) nor Group IV (average titre 2) shows such consistently high virulence (Table II). Avirulent strains are found in varying proportions in the other Type groups, in greatest number in Group IV (61 %), but there are none in Type I.

Two Type "A" strains (Lister) were discovered, one of high virulence, titre 4, and the other of low virulence, titre 1.

Though the figures cited are not large, on the whole the typing classification fails to distinguish virulent from avirulent strains, and as an index of pathogenicity is therefore unsatisfactory.

Virulence in relation to the Lesion.

The range of virulence covered by the 65 strains of pneumococci under review is wide, the M.L.D. varying from 50 cocci to 50 million cocci (titre 7 to titre 1). As it is not feasible to present all these strains in their order of virulence in the compass of a single table, they have been arranged in groups according to the lesion for which they were responsible. Table III shows them so arranged under the headings of lobar pneumonia, broncho-pneumonia, empyema, terminal pneumonia, etc., together with the type and virulence of each strain.

Some difficulty has occasionally been experienced in distinguishing the lobar type of pneumonia from broncho-pneumonia, and it is doubtful whether the two conditions are always susceptible of distinction, though the majority of cases fall naturally into one category or the other on both clinical and pathological grounds. Occasionally the classical picture of lobar pneumonia, with its acute onset, sustained high fever, and sudden crisis at the end of 5 to 7 days bringing dramatic relief to the patient, is not so well defined, and the absence of complete lobar consolidation or of crisis, or the irregularity and persistence of the fever and modified severity of the symptoms, suggests an approach to the broncho-pneumonic syndrome. Owing to the relatively crude methods of percussion and auscultation available for detecting consolidation in the lung, deeply seated pneumonia may be overlooked, while a lobe containing several patches of consolidation may be mistaken for a consolidated lobe. In this paper the term broncho-pneumonia has been employed in those cases where consolidation was incomplete or doubtful.

It is evident from the results recorded in the table (III) that these divisions on clinical grounds correspond closely with different grades of virulence in the infecting pneumococcus. The most virulent, lobar pneumonia, contains only those organisms of consistently high virulence, namely, titre 4–6, with an average of titre 5. The next group, broncho-pneumonia, consists of organisms of slightly lower virulence, titre 3–4, with an average of titre 3. The empyema strains present a range of virulence covered by both the previous groups together, titre 3–7, with an average of titre 5; an outcome not unexpected, since empyema commonly results from extension to the pleura of either a lobar or a broncho-pneumonic type of infection, the organism passing through the pleura and preserving its virulence unchanged.

Either type of pneumonia is apparently associated with pneumococci of constant and specific virulence, which is higher for lobar than for broncho-pneumonia but which is never lower than titre 3. As a general rule, therefore, pneumonia does not arise unless the invading organism possesses a virulence of titre 3 or more. This virulence may be regarded as critical, a *sine qua non*

Table III. *Showing the virulence of strains of pneumococci associated with different kinds of lesion.*

Lesion	Virulence titre	Type	Ref. No.
Lobar pneumonia	6+	I	E. 13
	6−	I	2579
	6−	I	P. 805
	5+	I	P. 787
	5+	I	83·26
	5+	IV	E. 27
	5	I	2811
	5−	IV	24·26
	4+	I	2374
Average Virulence Titre 5+.			
Broncho-pneumonia	4−	I	2305
	3+	I	2210
	3+	III	2174
	3+	IV	2140
	3	III	2229
Average Virulence Titre 3.			
Terminal pneumonia	5	III	3127
	*1?	IV	13·27
	*1−	S. saliv.	17·27
Post-operative pneumonia	3	IV	P. 826
	0	IV	P. 470
	0	S. saliv.	3·28
Nasal catarrh	*5−	IV	P. 943
	2+	IV	P. 820
	*0	IV	P. 1211
Conjunctivitis	*3?	III	3260
	*1?	IV	2906
	0	IV	2914
Panophthalmitis	5	III	3654
	5	IV	P. 505
	*4−	IV	2047

Lesion	Virulence titre	Type	Ref. No.
Empyema (pure primary infection)	7	I	P. 468
	6	I	2219
	6	I	P. 605
	6−	I	2744
	5+	I	3523
	5+	I	3556
	5+	I	3724
	5+	III	3453
	5	I	1797
	5	I	2579 *a*
	5	I	B. 6
	4+	I	1554
	4+	I	3482
	4+	III	2043
	4	I	1608
	*4−	"A"	P. 986
	3+	I	2374 *a*
	3+	IV	46·26
Average Virulence Titre 4+.			
Bronchitis (subacute to chronic)	*1?	IV	P. 1241
	*1?	IV	P. 1003
	*1?	IV	3625
	0	IV	P. 566
	0	IV	P. 699
	0	IV	P. 496
Average Virulence Titre less than 1.			
Meningitis	5+	I	P. 788
	4	I	9·27
	4	I	79·26
	4	I	3362
	4	I	2210 *a*
	2−	IV	1876
	1	"A"	1454
Arthritis (hip)	4+	I	2783
	4	I	3089
Peritonitis	*5−	IV	3291

Virulence titre = the logarithm of the reciprocal of the M.L.D. in c.c. of 18-hour broth culture, causing death within 48 hours of mice of standard age, weight and breed; *e.g.* if M.L.D. is $1/10^2$ c.c. the virulence titre is 2, and if M.L.D. is $1/10^x$ c.c. the virulence titre is x. Thus

Virulence titre 4 = both mice injected with 10^{-4} c.c. of broth culture died within 48 hours.

,, 4+ = the same, with the additional death of one mouse injected with the next dilution above.

,, 4− = both mice injected died, but not within the 48-hour period.

All the strains save those marked thus * gave a sharp end-point for their M.L.D. The mice either died within a few hours of the 48-hour period after inoculation, or remained well.

Those strains marked * failed to give such a sharp end-point. The mice either appeared well, or remained unwell, for several days, finally dying of pneumococcal septicaemia. Occasionally as much as 14 days elapsed before the event. The only conclusion possible at present is that these strains possess a somewhat different order of virulence, tending to set up a more chronic type of lesion in mice. The subject will be dealt with at length elsewhere.

for pulmonary infection, and such exceptions as there are prove the rule. For the only lesions in the lung associated with organisms whose virulence is below titre 3 are the terminal pneumonias and post-operative pneumonia. These are conditions arising in special circumstances and therefore requiring special consideration.

Persons contracting lobar pneumonia are usually in good health, whereas the victims of terminal pneumonia are already far advanced in disease. Their natural response to infection is so impaired that an organism which appears powerless to invade a healthy lung may in them cause a fatal lesion. Thus two out of the three strains isolated from cases of terminal pneumonia are practically avirulent, 13·27 and 17·27. Of these the first came from a patient, aged 72, in an advanced state of cachexia with carcinoma of the bladder; the second, 17·27, a salivary streptococcus, from a patient with congenital cystic disease of the kidneys, resulting in uraemia.

In the aetiology of pneumonia following surgical operation there are at least two unusual factors present. The deep, sometimes violent breathing during anaesthesia is apt to cause an abnormal intake into the lung of mucus laden with organisms from the pharynx. Especially is this prone to occur when there is excessive mucus secretion. The other factor is the condition of shock, often following abdominal and other operations, which tends to impair the various mechanisms of ciliary action, coughing, etc., that normally play an active part in removing foreign matter from the lung—Gaskell (1925) *et al.* Whatever the explanation, the fact is that two strains out of the three isolated from cases of post-operative pneumonia are avirulent. Both are from patients operated on for acute appendicitis; P. 470 is a Group IV pneumococcus, and 3·28 a salivary type of streptococcus, closely resembling a pneumococcus in its shape, cultural characters and fermentation of inulin. There are therefore indications that the bile-insoluble inulin-fermenting strains of salivary streptococci may set up pneumonia in the same circumstances as the bile-soluble organisms of low virulence, namely, in subjects already debilitated or collapsed. These pneumococci of low virulence were mostly of Group IV, whereas those causing lobar and broncho-pneumonia were all, save four, of the "fixed" types.

Another group of feebly virulent pneumococci, all belonging to Group IV, is collected under the heading of bronchitis (subacute to chronic). Bronchitis is not regarded as a pulmonary lesion but is included for sake of comparison. There are reasons for believing that salivary streptococci may also be responsible for bronchitis, playing the same part as the feebly virulent pneumococci.

With regard to lesions other than pulmonary lesions caused by pneumococci, such as nasal catarrh, conjunctivitis, and panophthalmitis, the data given in Table III lead to no very definite conclusions. Though the majority of these strains are not of the "fixed" types, some of them are highly virulent and might well cause pneumonia if they succeeded in reaching the lung in large enough dose. Of the three strains from patients with panophthalmitis, one, P. 505, was able to set up pneumonia and empyema when introduced into a rabbit's lung through the trachea, so that there is some justification for regarding them as potentially pneumonic strains.

In the meningitis group five highly virulent Type I strains are found, one feebly virulent Group IV and one Lister "A" strain of low virulence.

With the five virulent strains the infection appeared to be either primary to the meninges, or secondary to definite pulmonary infection. In the two cases, 1876 and 1454, however, in which the organisms were of lower virulence, the meningitis was secondary to suppuration in the accessory cavities of the skull, the frontal sinus and the middle ear, respectively—the infection having spread through the bone. All seven cases ended fatally, regardless of the differences in virulence of the strains.

The only cases not already mentioned are the two cases of arthritis and one of general peritonitis shown in the table, and two cases of abscess and one of empyema not shown in the table. The two arthritis cases occurred

Table IV. *Showing how virulence is maintained* in vivo *over considerable periods of time, by a strain isolated on two or more occasions from the same patient.*

No.	Reference No.	Date	Type	Virulence titre	Lesion
1	2579	9. x. 25	I	6 –	Lobar pneumonia
	2579 *a*	23. x. 25	I	5	Empyema
2	2374	13. vi. 25	I	4 +	Lobar pneumonia
	2374 *a*	6. vii. 25	I	3 +	Empyema
3	2210	12. iii. 25	I	3 +	Broncho-pneumonia
	2210 *a*	29. iii. 25	I	4	Meningitis
4	2305	8. v. 25	I	4 –	Broncho-pneumonia
	2305 *a*	12. v. 25	I	3	Secondary abscess in shoulder
	2305 *b*	2. vi. 25	I	4	
5	2407	3. vii. 25	IV	0	Secondary empyema (pus)
	2407 *a*	4. vii. 25	IV	1 – ?	„ (sputum)

For "Virulence titre" see Table III.

If variations in virulence occur they are of minor degree, never exceeding titre 1, and are unimportant when compared with the major differences found between different strains, *vide* Table III.

in children and involved the hip-joint. An attempt was made to recover pneumococci from the throats of these patients, but was unsuccessful probably because the throat swabs were taken too late. One of the cases arose in a Type I epidemic of pneumonia, and no doubt the pneumococcus gained entry by the respiratory tract. Of the two cases of abscess not recorded in the table, both were secondary to pneumonia and appeared as complications during convalescence. The case of empyema not recorded arose as a terminal event in the course of Pott's disease, the tuberculous abscess having burst into the pleura and become secondarily infected with a Group IV avirulent pneumococcus.

CHANGES OF VIRULENCE *IN VIVO*.

The level of virulence maintained by the strains in each group of pulmonary lesion has been shown to be remarkably constant, in contrast with the variation in the types of organism. Further, the virulence of a given strain *in vivo* appears to remain stationary over considerable periods of time. Observations made on strains recovered at different times from the same patient disclose little change in the virulence, *vide* Table IV. Five different cases are cited, and in each case the organism isolated on the second or third

occasion is of the same type as the first organism, and differs only slightly in virulence from it (by titre 1 at most). Such a difference is negligible when compared with the major differences found to occur between different strains, and it is reasonable to assume that the organism recovered in the second and third instance in each case was identical with the one first isolated. In cases 1, 2 and 5 the organism probably passed direct from the lung through the pleura, but in cases 2 and 4 it more likely reached the secondary focus by metastasis *via* the blood stream.

Discussion.

The virulence for mice of 65 strains of pneumococci recovered at random in routine practice from a great variety of lesions has been measured. Comparison of these figures has shown that virulence is of major, the "type" of organism of minor, importance in determining the ability of a strain to produce lesions in the lung. The grade of virulence affords a means of grouping the various strains, their different grades of virulence corresponding closely with different types of pulmonary lesion. Lobar pneumonia is caused by the most virulent strains (titre 4–6), broncho-pneumonia by strains of rather lower virulence (titre 3–4), and empyema by strains whose grade of virulence is characteristic of both types of pneumonia (titre 3–7).

The virulence associated with each type of lesion is remarkably constant, and appears to be specific to the lesion. Moreover, there is evidence that the virulence of strains *in vivo* remains constant over a considerable period of time, for weeks at least.

A close parallelism exists between these lesions in man and those produced experimentally in the rabbit by Gaskell (1925). For the virulence in the infecting pneumococcus required to set up lobar pneumonia in the rabbit is the same as in man; and the parallel extends to broncho-pneumonia and to empyema. Below this virulence no pulmonary lesion is produced in either a healthy man or rabbit.

The effect of gross interference with the health of the subject, however, such as occurs in advanced wasting diseases, may be to allow a strain of considerably lower virulence to start a pneumonic process in the lung. Other abnormal factors, having similar effects, may be introduced by the administration of an anaesthetic, during which infected mucus may be drawn from the pharynx into the lung, and the condition of shock following surgical operation may delay the cleaning process which normally goes on in the air-passages. Under such circumstances a terminal pneumonia or a post-operative pneumonia may be caused by pneumococci of very low virulence.

Apart from pulmonary lesions, pneumococci found in nasal catarrh, conjunctivitis, bronchitis, etc., are generally not so virulent as those causing lobar and broncho-pneumonia, nor are they so often of the "fixed" types. For instance, all those associated with bronchitis are comparatively avirulent and belong to Group IV. Such strains appear to be closely related to the

salivary streptococci, which possess all the characters of pneumococci except bile-solubility, even agglutinating with pneumococcal Type III serum, and are often found to be the predominant organism in such conditions. Nevertheless there do occur a number of highly virulent strains, for instance, in panophthalmitis, which most probably can cause pneumonia in man, since one of them actually did so in rabbits.

Correspondence between the virulence of the organism, the kind of lesion and the course of the illness in the two species, argues that pneumonia in man and artificial pneumonia in the rabbit are fundamentally similar in their pathology. Unlike bronchitis, nasal catarrh, conjunctivitis, etc., which are more often due to infection with feebly virulent organisms, pneumonia is a disease caused by pneumococci of high virulence only, which reach the lung *via* the air-passages. More links must be forged before the chain of evidence is complete. For instance, the organism which sets up pneumonia in man should set up a like lesion in the rabbit—a result which is the natural outcome of the discovery of parallel virulence. Its demonstration must, however, be left for description in another paper.

That lobar pneumonia and broncho-pneumonia arising in previously healthy subjects are not only air-borne, but also infectious diseases, is a suggestion that cannot be ignored. Nurses and others in contact with the disease have been found commonly to harbour in their throats organisms of the same type as the disease-producing strain, whereas persons not in contact do so only very rarely—Avery and Dochez (1915), Stillman (1916 and 1917). Is the infection spread by contact, with the possible intervention of "carriers" and with a low incidence of the disease among "contacts," comparable to the low incidence in cerebro-spinal meningitis—Foster and Gaskell (1916)? The answer is most likely in the affirmative, though the evidence is not yet complete.

CONCLUSIONS.

1. The virulence of 65 freshly isolated strains of pneumococci has been compared by means of their M.L.D. for mice of standard age, weight and breed.

2. There is a certain specific minimal virulence below which pneumococci appear unable to infect the healthy human lung. This minimum is fairly high (M.L.D. approx. 500,000 cocci).

3. Lobar pneumonia, broncho-pneumonia and empyema arising in previously healthy subjects are caused by strains of high virulence. Lobar pneumonia is caused only by strains of the highest grade of virulence (M.L.D. approx. 5000 cocci); broncho-pneumonia by strains of a rather lower grade of virulence (M.L.D. approx. 500,000 cocci); empyema by strains whose virulence is characteristic of either lobar or broncho-pneumonia.

4. There is probably no sharp line of demarcation between lobar pneumonia and broncho-pneumonia, the one grading imperceptibly into the other; but since the lobar type of pneumonia is caused by the strains of higher

virulence the reaction is sharper, the symptoms are more severe and the mortality is higher than in broncho-pneumonia.

5. Pneumonia in subjects whose health has been weakened by advanced disease, or temporarily by shock, may be produced by pneumococci, and sometimes by salivary streptococci, of very low virulence.

6. Bronchitis, conjunctivitis, nasal catarrh and possibly other minor infections, are commonly caused by organisms of low virulence similar to those causing pneumonia in debilitated subjects.

7. The avirulent strains of pneumococci appear to be closely related in their pathogenic disposition to certain salivary streptococci. These they also resemble in their morphology, cultural characters, fermentation of inulin and immunity reactions.

8. The virulence associated with lobar pneumonia in man is the virulence requisite in a strain for the production of lobar pneumonia experimentally in the rabbit; and there is a like parallel for broncho-pneumonia and empyema. The pathology of pneumonia in the two species is almost certainly the same.

9. The "Type" of pneumococcus is of secondary importance in determining its ability to cause pneumonia.

10. The "fixed Types," particularly Type I strains, show a higher virulence on the whole than the Group IV strains; but Group IV cannot be considered the avirulent, or saprophytic, group, since several of its members (40 per cent.) are highly virulent and are not infrequently the cause of pneumonia.

11. Type I accounts for more than two-thirds of the cases of lobar pneumonia, broncho-pneumonia and empyema; and all the Type I strains isolated are probably capable, by virtue of their high virulence, of giving rise to pneumonia. No Type II strains have been recovered during the years 1924–27. Two strains have occurred which exhibit specific agglutination with Lister's Type "A" serum.

I wish again to record my gratitude to the British Medical Association for their generous support. I am also deeply indebted to Dr J. F. Gaskell, at whose instigation the work was undertaken, and by whose advice I have constantly profited; also to Prof. W. E. Dixon, in whose laboratory all the animal experiments were carried out. Prof. Simon Flexner and Dr R. R. Armstrong very kindly provided the Type sera employed in the research, and Dr St John Brook the National Type Cultures.

REFERENCES.

ARMSTRONG, R. R. (1921). The Serological Characters of Disease-producing Pneumococci. *Brit. Med. J.* **1**, 259.

—— (1922). Application of the Absorption of Agglutinin Test to the Serological Study of Pneumococci. *Brit. J. Exp. Path.* **3**, 287.

—— (1923). The Correlation of Clinical Manifestations and Serological Types in Pneumococci. *St Bartholomew's Hosp. Rep.* **56**, 85.

AVERY, O. T. and DOCHEZ, A. R. (1915). The Occurrence of Carriers of Disease-producing Types of Pneumococci. *J. Exp. Med.* **22**, 105.

FOSTER, M. and GASKELL, J. F. (1916). *Cerebro-spinal Fever*, pp. 123 *et seq.* (Camb. Univ. Press.)

GASKELL, J. F. (1925). Experimental Pneumococcal Infections of the Lung. *J. Path. and Bact.* **28**, 427.

GLYNN, E. E. and DIGBY, L. (1923). Bacteriological and Clinical Observations on Pneumonia and Empyemata, with Special Reference to the Pneumococcus and to Serum Treatment. *Med. Res. Council, Special Report Series*, No. 79.

GRIFFITH, F. (1923). The Influence of Immune Serum on the Biological Properties of Pneumococci. *Reports on Public Health and Medical Subjects*, No. 18, p. 9.

—— (1928). The Significance of Pneumococcal Types. *J. Hygiene*, **27**, 113.

LISTER, F. S. (1913). Specific Serological Reactions with Pneumococci from Different Sources. *Publications of the S. African Inst. for Med. Research*, Johannesburg, Dec. 22, **1**.

REIMANN, H. A. (1925). Variations in Specificity and Virulence of Pneumococci during Growth *in vitro*. *J. Exp. Med.* **41**, 599.

STILLMAN, E. G. (1916). A Contribution to the Epidemiology of Lobar Pneumonia. *Ibid.* **24**, 651.

—— (1917). Further Studies on the Epidemiology of Lobar Pneumonia. *Ibid.* **26**, 513.

WHITTLE, C. H. (1927). The Determination of Virulence of Pneumococci Freshly Isolated from Human Lesions. *J. Path. and Bact.* **21**, 305.

—— (1928). On the Identification of Pneumococci, and the Tests Employed for Distinguishing them from Streptococci. *J. Hygiene*, **27**, 200.

(*MS. received for publication* 1. III. 1928.—Ed.)