A STUDY OF PNEUMOCOCCI AND ALLIED ORGANISMS IN HUMAN MOUTHS AND LUNGS AFTER DEATH.

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Our studies have been confined especially to a determination of the prevalence of the pneumococcus in normal lungs, and in lungs which presented various lesions. At the same time, we have tried to determine, by experimental methods, how justly one may draw inferences with regard to the flora of the living lung from cultural findings after death.

Numerous investigations have shown that the pneumococcus is more or less constantly present in a variety of pulmonary lesions, especially in lobar pneumonia. The pneumococcus has been isolated from a considerable proportion of apparently healthy lungs, both human and animal, that have been examined after death. A similar micro-organism, or one closely related to it, has been found in the mouths of many healthy human beings.

The question of the presence of bacteria in normal lungs has long been an urgent one, because of its obvious bearing upon the determination of the occurrence of the infectious diseases of this organ. If organisms are present in all lungs, it may be supposed that the pneumococcus will be more frequently found in the lungs of patients exposed for some time to a hospital atmosphere. This point, too, we have tried to decide, and for this reason we have classified our cases into the following three groups, viz:

I. Patients dying outside of hospital.

II. Patients dying in hospital within twenty-four hours of admission.

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III. Patients dying in hospital twenty-four hours or longer after admission.

This report embodies the results of the bacterial examination of a series of forty-two (42) human lungs. The cases were taken at random, so as to comprise a variety of lesions. There were included cases which died in the wards of Bellevue Hospital, and a lesser number of coroner's cases, which died either outside the hospital or in the hospital within twenty-four hours after admission.

These lungs were selected from subjects examined within twenty-four hours after death. The bodies were removed from the wards, usually within an hour after death, and kept in cold storage until examination.

METHODS EMPLOYED IN THE STUDY OF THE BACTERIA IN THE MOUTH AND LUNGS.

Isolation.—The isolation of the organisms studied was carried out in the following manner: The lung to be cultivated was clamped before removal, by a large hysterectomy clamp, which firmly occluded the large vessels and bronchus. This was done to prevent, as far as possible, the aspiration of fluid or mucus from the trachea, during the manipulation of removing the lungs. The surface of the lung was thoroughly seared with a large knife over the posterior portion of the lower lobe. The juice was obtained in the usual manner, by stabbing the sterile area with a heated Nuttall spear, and was then thinly streaked on glycerine-agar plates. The fluid suspension of material from the lungs for the inoculation of the mice was obtained as follows: With sterile forceps and scissors, a large piece of lung was excised from the seared area and cut into small pieces in a sterile Petri dish; in cases where the juice expressed was insufficient a little broth was added. From 0.5 to 1.0 c.c. was inoculated into the skin of the back. In the majority of cases white mice were used, but, owing to the difficulty of obtaining a sufficient number, we were compelled (in a few instances which are noted) to use colored or spotted mice.

From the lung plates, after from twenty-four to forty-eight hours, sub-cultures were made on Loeffler's blood-serum from pneumococcus- or streptococcus-like colonies; usually as many as half a dozen were transplanted.

From the heart's blood of mice dying after inoculation, streak plates were made on glycerine-agar, and the colonies which had developed after from twenty-four to forty-eight hours were sub-cultured on Loeffler's blood-serum. In case the colonies varied in appearance, several sub-cultures were always made from each type.

For the isolation of the pneumococcus from saliva we have depended almost wholly upon the subcutaneous inoculation of mice with a small amount of mucus from the mouth. This was obtained at autopsy, on a cotton swab, which was then shaken in broth and the suspension injected. The subsequent proceedings were identical with those above described for the lungs. Glycerine-agar plates were made in a few instances, but, on account of the difficulty encountered in obtaining satisfactory plates, this was not done as a routine.

Morphology.—The morphology of the bacterial flora of the lung was studied in cover-slip preparations and in sections of lung tissue. As a routine stain for the demonstration of capsules, the two staining methods as described by Hiss [2] were employed; in smears from the lung tissue, a Gram preparation was also made. Sections were stained for bacteria by the Gram-Weigert method.

Cover-slip preparations from the heart's blood of mice, from typical colonies on plates, from the primary sub-cultures on Loeffler's medium, were examined as a routine, and in many instances from the transplantations on different media.

Determination of Cultural Characters.—For the study of cultural characteristics, transplants were made from the primary Loeffler sub-cultures upon the following media: broth, gelatine, glycerine-agar slants, litmus-milk, Hiss' 1 % inulin-serum water (1 : 3). In addition, the fermentative activities of a certain number of what we may for the present call the different

[2] *Journal of Experimental Medicine*, 1905, vi, 317.

strains of the pneumococcus, as well as the streptococcus, were studied on a series of sugar media containing dextrose, lactose, maltose, saccharose, mannit, or glycogen, and on dextrin and soluble starch.[3]

Determination of Virulence.—This was limited to the inoculation of mice with half or whole cultures, grown for twenty-four hours on glycerine-agar slants. Unfortunately, we were not in a position to determine accurately the virulence of the various strains, owing to the insufficient supply of mice at our disposal. Because of this scarcity we rarely were able to ascertain the virulence of the organism in fresh isolations.

DESCRIPTION AND CLASSIFICATION OF THE PNEUMOCOCCI, STREPTOCOCCI, AND ALLIED FORMS, FOUND IN THE LUNGS AND MOUTHS.

In consonance with the opinions of more recent observers, we believe that the older criteria relied upon for the differentiation of pneumococci and streptococci are insufficient. Even the fermentative activities upon which so much stress has recently been laid have not been, in our hands, an infallible guide, especially with reference to the identification and classification of the intermediate types of these two closely related cocci, of which many have been encountered.

We must likewise take exception to the older and perhaps still widely prevalent view, that streptococci, unlike the pneumococci, may not produce a bacteriæmia in mice, as in many cases we were able to isolate streptococci in cultures from the heart's blood of mice taken immediately after death.

In order to describe the bacteria we have encountered during the course of this work we have been forced, somewhat against our will, to adopt an arbitrary classification founded mainly upon

[3] The sugars were obtained from Merck. The inulin employed by us in the earlier determinations was obtained from Merck. This inulin was found highly unsatisfactory, from the presence of resistant spores. All our later work was done with Kiliani's inulin, "Eimer and Amend." Many of our earlier cultures were tested again upon this same inulin. Kahlbaum's soluble starch was used for the starch-serum water, in the strength of two per cent.

the variations in morphological and physiological characters. The most important and constant of these are the presence or absence of capsules, and the fermentation or non-fermentation of inulin. Our grouping of these cocci is as follows:

GROUP I.

Typical pneumococci.—By this, we designate those diplococci which ferment inulin with acid formation, and coagulation of the serum; which possess readily stained capsules, not only in the blood of mice, but on various media; which give a diffuse cloudiness in broth, and grow as fine, colorless, delicate, translucent colonies on glycerine agar, and which are virulent to mice. We have met with a number of organisms which, although fermenting inulin with slight acid production, do not coagulate the serum. This, we consider, indicates, in general, feeble growth.

GROUP II.

Streptococcus mucosus.—By this term, we refer to a series of organisms which differ from the typical pneumococcus in their abundant, moist, or mucous growth upon various solid media and in the more constant production of chains upon media, but which resemble the pneumococcus in possessing a constantly demonstrable capsule, in their fermentative activity, and in their virulence to mice.

In conformity with the observations of other observers, we have found that the capsules of these diplococci are more easily demonstrable than those of the typical pneumococcus, and that there are certain variations in the structure of the capsules, which we have not observed in those of the pneumococcus. We have found that the cocci of this group grow more constantly and abundantly at room temperature in gelatin.

We have not as yet been able to decide definitely, whether the group constitutes a distinct variety of the pneumococcus, or whether the mucous character of the growth and chain formation is dependent upon variations in the media. Thus we have found that on dry Loeffler's serum tubes the growth of these organisms may be dry, whereas on wet tubes it tends to be mucous-like in character. We have also seen organisms resembling the pneumococcus, after passage through mice, assume a viscid character of growth. In fact, there seem to be numerous gradations between these two types in this respect.

GROUP III.

Diplococci resembling pneumococci which possess no demonstrable capsule.—No capsules have been demonstrated in these diplococci, either in the heart-blood of mice, or on culture media after repeated examination. They produce acid in inulin, but do not coagulate the serum. In all other respects, they resemble pneumococci.

GROUP IV.

Diplococci resembling streptococci which possess no demonstrable capsule.—No capsules have been demonstrated in these diplococci, either in the heart-blood of mice, or on culture media. They are active inulin fermenters. These cocci, although fermenting inulin with acid production, have greater points of similarity to the streptococcus than to the pneumococcus. They grow abundantly in artificial media, produce clouding, or flocculi and granules in broth, grow readily at room temperature, and show a marked tendency to chain formation.

GROUP V.

Typical streptococci.—These have no capsules, do not ferment inulin with acid production, and grow readily at room temperature. They produce clouding in broth, or form granules or flocculi adherent to the sides of the tubes.

SOURCES OF ERROR IN TECHNIQUE.

It is obvious that there are many possibilities of error in the isolation of pneumococcus-like organisms from the lungs by methods above detailed. On account of the similarity in the colonies on glycerine-agar, and even on ascitic-agar, there is always difficulty in distinguishing on inspection pneumococcus from short-chained streptococcus colonies, and in consequence the pneumococcus, though present in small numbers, may escape detection, for it is obviously not practical to sub-culture more than a limited number of suspicious colonies.

Overgrown plates are another but not a frequent source of trouble. The organisms responsible for this are not only those which are introduced through natural errors in technique, but also those which we have found more or less constantly present in the lungs cultivated after death, as verified by a thorough examination of cover-slip preparations from the lung tissue.

Again, another source of error lies in the natural insusceptibility of white mice, and even more so of certain strains of colored mice, to infection by pneumococci. Thus it has been found by us that colored mice are more resistant to infection than white mice when inoculated with similar doses. Moreover, it has been found that in some of our cases even a white mouse does not succumb to the inoculation of a comparatively large amount of expressed lung juice, although cover-slip preparations

of the lungs have shown typical capsulated diplococci, positive to Gram. For this reason, so far as it has been possible, we have inoculated two mice from each case.

Still another though less frequent and serious difficulty is that encountered in the isolation of pneumococci in streak agar plates from the heart blood of mice dying of mixed infection. In a certain number of cases we have found Bacillus mucosus capsulatus to be the predominating organism; in other cases, non-capsulated cocci which we have not as yet identified. In a certain number of cases also, though pneumococcus-like organisms were present in smears from the lung, the mice inoculated died from streptococcus, Bacillus mucosus capsulatus, or other infections. On the other hand, we have encountered capsulated cocci in the heart's blood of mice, as well as upon our lung plates, which failed to grow on transplantation upon glycerine-agar or Loeffler's blood-serum.

With this brief introduction, we can now present more readily the result of our work largely in tabulated form. The tables which follow must be briefly described.

The first column of the tables, headed "Case Number," indicates the number of the case in our series. The next column, the "Accession Number," refers to the entry number of each case in the autopsy accession book of the hospital. The Roman numerals I, II, III, IV, and V are those we have used to designate the five groups into which we have divided the pneumococci, streptococci, and the cocci allied to them.[4] The addition sign (+) or the zero sign (o) opposite each case indicates that a coccus of this group has or has not been isolated.

[4] I.—Pneumococcus.
II.—Streptococcus mucosus.
III.—Diplococci without capsules, which ferment inulin only with acid production.
IV.—Diplococci without capsules, which are active inulin fermenters, and which closely resemble streptococci.
V.—Streptococci.

PNEUMOCOCCI, STREPTOCOCCI, AND ALLIED MICRO-ORGANISMS IN MOUTHS AFTER DEATH.

In fourteen cases the mouths were cultivated by the methods sufficiently described above. We append the analysis of our findings:

TABLE I.

ANALYSIS OF PNEUMOCOCCI, STREPTOCOCCI, AND ALLIED MICRO-ORGANISMS, ISOLATED FROM THE MOUTH AFTER DEATH.

	Case No.	Accession No.	Group I.	Group II.	Group III.	Group IV.	Group V.	Unidentified.
	1.	82	+	o	o	o	o	(?)
	2.	98	o	o	o	o	+	
	3.	105	o	o	o	o	+	
	4.	110	+	o	o	o	o	
	5.	153	o	o	o	+	o	
	6.	164	o	o	o	o	+	
	7.	165	+	o	o	o	o	
	8.	166	+	o	o	o	o	
	9.	167	o	o	o	o	o	+
	10.	170	o	o	o	o	+	
	11.	172	o	o	o	o	o	+
	12.	191	+	o	o	o	+	
	13.	199	o	o	+	o	o	
	14.	233	o	o	o	o	o	
Total	14 cases		5	o	1	1	5	2

The pneumococcus, as shown by Table I, was isolated five (5) times, the streptococcus five (5) times, and intermediate organisms twice. Of these twelve cases, all but three were in the hospital over twenty-four hours. Two died with lobar pneumonia; in one of these, the mouse injected with mouth secretion died of streptococcus infection, and this organism, in pure culture, produced a bacteriæmia fatal within twenty-four hours in a mouse inoculated with three-fourths of a glycerine-agar growth twenty-four hours old.

The virulence of the cocci isolated was tested in eight (8) cases. Three (3) of these were pneumococci, four (4) were streptococci, and one (1) Group IV. One of the streptococcus cultures proved to be non-virulent. All the others killed the

mice in from eighteen to forty-eight hours, partial or whole cultures being inoculated.

All the mice inoculated with mouth secretion died, in most cases, within twenty-four hours. Mice inoculated with the secretion from two cases died with infections other than pneumococcus or streptococcus. From a third case, one of the mice inoculated died after four (4) days, no organisms being recovered in smears or by culture; the second mouse of the same case remained alive.

These few observations seem to show that the streptococci isolated from the mouth were quite as virulent as the pneumococci. The pneumococci obtained from two (2) cases not dying of lobar pneumonia were found to be quite as virulent as those obtained from pneumonic lungs post-mortem. Further conclusions, however, as to the general virulence of the pneumococci obtained from the mouth do not seem to be justified.

We have made no attempts to determine the frequency of pneumococci in the mouths of ward patients or attendants on account of an insufficient supply of mice.

PNEUMOCOCCI, STREPTOCOCCI, AND ALLIED BACTERIA ISOLATED FROM THE LUNGS AFTER DEATH.

Forty-two (42) cases were examined. The analysis of our findings follows in tabulated form (Table II).

Table III shows the number of cases in which the various groups of cocci have been found, either alone or in association with the other groups.

We are now in a position to analyze the facts presented above, in Table IV, which requires a brief explanation. The cases cultivated in this research have been divided into three classes, according to whether the lungs were normal in the gross or presented the lesions of lobar pneumonia, or were the seat of a variety of other lesions. Each of these classes has again been divided into three classes, viz., those cases dying outside the hospital, those dying in the hospital within twenty-four hours of admission, and those dying twenty-four hours or more after admission.

TABLE II.

ANALYSIS OF THE DIPLOCOCCI ISOLATED FROM THE LUNGS.[5]

Case No.	Accession No.	Group I.	Group II.	Group III.	Group IV.	Group V.	Unidentified.
1.	43	o	o	o	o	o	
2.	45	o	o	o	o	+	
3.	46	o	o	o	o	+	
4.	49	+(?)[6]	o	o	+	+	
5.	52	o	o	o	+	o	
6.	54	o	o	o	+	o	
7.	63	+	o	+	+	o	
8.	68	+(?)	o	o	o	o	
9.	70	+(?)	o	o	o	o	
10.	71	o	+	o	o	o	
11.	82	+	o	+	o	o	
12.	98	o	o	o	o	+	
13.	102	o	o	o	o	+	
14.	105	+(?)	o	o	o	+	
15.	110	+	o	o	o	o	
16.	113	o	o	o	o	+	
17.	114	+(?)	o	o	o	+	
18.	123	o	o	o	o	o	+
19.	140	+	+	+	o	o	
20.	147	o	o	o	o	o	
21.	149	o	o	o	+	o	
22.	153	o	o	+	o	o	
23.	160	o	+	o	o	o	
24.	164	o	o	o	o	+	
25.	165	+	o	o	o	+	
26.	166	+	o	o	o	+	
27.	167	o	o	o	o	+	
28.	170	+	o	o	o	+	
29.	172	+	o	o	o	o	
30.	175	o	o	o	o	+	
31.	191	+	o	o	o	o	
32.	199	o	o	+	o	+	
33.	C I	+	o	o	+	o	
34.	C II	o	o	o	+	+	
35.	C III	+	o	o	o	o	
36.	C IV	+	o	o	o	o	
37.	C V	o	o	o	o	+	
38.	C VI	o	o	o	o	+	
39.	C VII	+	o	o	o	o	
40.	233	+	o	+	o	+	
41.	237	+	o	o	o	o	
42.	258	+	o	o	o	o	

[5] See explanation of Table I, p. 456.

[6] The cocci of Group I which are marked (?) were not absolutely identified as pneumococci, but they in all probability belong to the group, and they have therefore been included.

TABLE III.

ANALYSIS OF THE DIPLOCOCCI ISOLATED FROM LUNGS.

Group of Organisms.[7]	No. of Times Found.	Accession No. of Cases.
I alone,	10	68, 70, 110, 172, 191, C III, C IV, C VII, 237, 258.
II "	2	71, 160.
III "	1	153.
IV "	3	52, 54, 149.
V "	10	45, 46, 98, 102, 113, 164, 167, 175, C V, C VI.
I + II	0	
I + III	1	82.
I + IV	1	C I.
I + V	5	105, 114, 165, 166, 170.
II + III	0	
II + IV	0	
III + V	0	
III + V	1	199.
IV + V	1	C II.
I + III + IV	1	63.
I + II + III	1	140,
I + IV + V	1	49.
I + III + V	1	233.
Unidentified.	2	123, 147.

The data presented in Table IV may be thus briefly reviewed:

I.—NORMAL LUNGS.

a. Two cases dying outside of hospital.—In one, the pneumococcus was isolated; in the other, only a streptococcus.

b. Four cases dying within twenty-four hours of admission.—In these (63, 68, 153, 223) the pneumococcus was isolated three times; one of these cultures (68), however, may be considered questionable.

It is interesting to note that likewise in three of the cases organisms of Group III (non-capsulated inulin fermenter) were found, and in two of the cases streptococci (Group V).

c. Eight cases, dying twenty-four hours or more after admission.—The pneumococcus was obtained in two cases: in 166, where it was associated with streptococcus (Group V); and in 258, where it occurred alone. In two cases (71 and 160), Streptococcus mucosus (Group II); in one case (149), Group IV; in four cases (98, 166, 167, C V.), streptococci (Group V).

The pneumococcus, if 68 be called a true pneumococcus, was thus isolated five times. It is, perhaps, noteworthy, that pneumococcus was isolated from the cases dying outside, and those less than twenty-four hours in hospital, in four out of six cases, 66 %, whereas it was isolated in only two out of eight cases which had been in the hospital over twenty-four hours, 24 %.

[7] See explanation of Table I.

TABLE IV.

ANALYSIS OF THE CASES, ACCORDING TO THE GROUPS OF DIPLOCOCCI, AND THE LESIONS FOUND IN THE LUNGS AT AUTOPSY AND ALSO ACCORDING TO THE LENGTH OF TIME OF THE CASES IN THE HOSPITAL.

	Normal.						Lobar Pneumonia.						Other Pulmonary Lesions.						Total.					
	No. of Cases.	Group I.	Group II.	Group III.	Group IV.	Group V.	No. of Cases.	Group I.	Group II.	Group III.	Group IV.	Group V.	No. of Cases.	Group I.	Group II.	Group III.	Group IV.	Group V.	No. of Cases.	Group I.	Group II.	Group III.	Group IV.	Group V.
Not in Hospital.....	2	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	1
In Hospital 24 hours or less.	4	3	0	3	1	1	3	1	1	2	0	1	2	2	0	0	1	0	9	6	1	5	2	2
In Hospital over 24 hours..	8	2	2	0	1	4	9	5	0	0	0	6	13	6	0	1	4	6	30	13	2	1	5	16
Total.....	14	6	2	3	2	6	13	7	1	2	0	7	15	8	0	1	5	6	42	21	3	6	7	19

SUMMARY OF TABLE IV.

Total number of cases cultured..... 42
Micro-organisms obtained in..... 41
Group I..... 21 (5 doubtful).
" II..... 3
" III..... 6
" IV..... 7
" V..... 19
Unidentified..... 2

II.—LOBAR PNEUMONIA.

Thirteen (13) cases, one dying outside the hospital, three (3) within twenty-four hours of admission, and nine (9) after a residence in hospital of twenty-four hours or over. The pneumococcus was isolated from seven of these cases; this includes one non-inulin fermenter (114), which, however, exhibited a capsule after the second passage through mouse, the culture having been taken from the pneumonic lobe.

Cultures were made in seven of the thirteen cases from the consolidated lobe; in two, from the non-consolidated. Of the remaining four (4) cases, which showed organization of the exudate (so-called organizing pneumonia), cultures were made from the involved lobe three (3) times; from the uninvolved lobe, once.

From the seven lobes showing a typical pneumonia with exudate, in three cases (140, C VII, 237) typical pneumococcus (Group I) was obtained; once (140) associated with Streptococcus mucosus (Group II); in two cases (105, the organism growing poorly, and 114, above mentioned) two typical pneumococci were isolated, associated with streptococci (Group V); in another case (199) Group III, and also a streptococcus, Group V; in another (113) Group V was isolated, no other organisms being found in this case.

Of two cases cultivated from the uninvolved lobes, one (110) gave a typical pneumococcus; the other (147) showed B. mucosus capsulatus in pure culture from the lungs in plates, and from the mouse in plates, no pneumococci having been found in the cover-slip preparations made from the heart's blood of mouse.

In three cases where the lobes with organizing pneumonia were cultured, in one (43) no bacteria were found in plates, the single mouse inoculated remaining alive; in two cases (46 and 102) streptococcus (Group V) alone was isolated.

In one case of organizing pneumonia (165), in which the involved lobe was cultured, a pneumococcus (Group I) and a streptococcus (Group V) were isolated.

III.—LESIONS OTHER THAN LOBAR PNEUMONIA.

Of the fifteen remaining cases, none died outside the hospital; two were in hospital less than twenty-four hours; in one of these (C I) pneumococcus and an organism of Group IV were found; in the other (C III) pneumococcus alone was found.

Of the thirteen cases over twenty-four hours in hospital, the pneumococcus was found in four cases; in two cases (49 and 70) organisms, probably pneumococci (Group I), were obtained. In Case 49, organisms of Groups IV and V were also isolated; in 70, no other organisms were found. In one case (123) an unidentified non-capsulated diplococcus, which did not grow on transplantation from the lung plates, was obtained; the mouse inoculated with lung tissue remained alive.

From the tabulated summary of Table IV we see that the total number of cases cultured was forty-two, and that in all but two

(2) cases micro-organisms were obtained. From these cases, pneumococci and streptococci were obtained in practically similar percentages—that is, in 50 %. These results, as far as the pneumococcus is concerned, agree with those obtained by Beco, 50 % pneumococci and 45 % streptococci in human lungs.[8] Boni,[9] in lungs of pigs, has also obtained similar percentages. Dürck,[10] in pneumonic lungs of children, obtained pneumococcus in cases of lobar pneumonia, some of which were organizing or unresolved pneumonias, in seven out of thirteen, 52 %, streptococci being found in the same proportion.

Discussion of the Significance of the Data above Summarized.—A consideration of these facts suggests a number of interesting questions: Are the pneumococci present in the exudate of lobar pneumonia similar to the pneumococci which have been found in the mouths of normal persons, presumably as harmless inhabitants? If so, they reach the lungs either through the lymphatics or the vascular channels at some time during life, or by inhalation, lodging there as harmless saprophytes until some as yet unknown change in their host causes them to acquire increased virulence. A second possibility is that pneumococci of greater virulence than those found in the mouth are inhaled with the air current during life, determining the onset of the disease. However, it is still an open question whether pneumococci are present in the lungs of normal persons during life. May not their presence in normal lungs, as found by post-mortem examinations, be explained by a terminal septicæmia, such as has been established for the streptococcus and other micro-organisms, or do the pneumococci of the mouth gain access to the air vesicles with the saliva, either aspirated during the death agony or, later, by gravitation consequent upon the manipulation of the body after death, in its transportation from the wards to the morgue?

These problems complicate the interpretation of the various factors concerned in the production of lobar pneumonia. With

[8] Beco, *Archiv. d. la medecine expérimentale*, 1889, xi, 317.
[9] Boni, *Deutsch. Arch. f. klin. Med.*, 1901, Bd. 69.
[10] Dürck, *Deutsch. Arch. f. klin. Med.*, 1897, Bd. 58.

the more theoretical aspects of the question, such as the disturbance in balance between the host and the inciting agent of the disease, we have not attempted to cope, except in so far as we have roughly determined the virulence for mice of the organisms isolated in our mixed series of cases.

The statistics obtained by the investigators mentioned above and by ourselves might lead one to the inference that bacteria of the pneumococcus or streptococcus group exist during life in practically all lungs, whether normal or diseased. But, as we have already suggested, grave theoretical objections may be urged against such a conclusion. Though we have been able to obtain these organisms in 97 % + of our cases, the possibility, as suggested above, that the pneumococci so frequently present in the mouth may reach the lungs by aspiration during the death agony, or after death, during the transportation of the bodies from the wards to the morgue, is no fanciful one.[11]

The mouth and nose are frequently found at autopsy filled with fluid admixed with frothy and bloody fluid, or vomitus. It is quite evident, under such conditions, that, by mere force of gravity and the fluid communication existing between the upper and lower air-passages, micro-organisms may find their way from

[11] Because of its possible bearing upon the gravitation of fluid from the buccal cavity to the lungs, it seems worth while to describe in detail the various manipulations to which the body is subjected during the interval between death and post-mortem examination. After being formally pronounced dead by a member of the house staff, the body is rolled in a shroud, the jaw supported by a four-tailed bandage, and cotton plugged into the mouth and other orifices. Within an hour or less, the cadaver is ready for transportation to the morgue. It is lifted by the shoulders and heels, to a four-wheeled truck, which has been rolled to the bedside. During this manœuvre, with the sagging of the body the head is of necessity at a higher level than the thorax. From the ward, the body is wheeled directly to the morgue,—a distance of about 150 yards. Then again it is lifted from the truck to a wooden frame, which is placed upon the floor next to the carriage, and lying upon this, it is raised, feet foremost, to a compartment in the cold-storage chamber. Here it remains until removed to the autopsy room. In this final handling of the body, the wooden tray is slid from the compartment, the head lowest, and the body is carried upon this wooden frame to the adjacent room. The body is then rolled upon its side, abruptly jolted, and the head raised and lowered several times. The entire proceeding, therefore, involves considerable disturbance of the body before the necessary examinations are made.

the mouth to the small air spaces; or, again, by the compression of the chest wall in moving the body a false respiratory excursion may take place, causing the replacement of the air in the lungs by fluid from the mouth and trachea. If this takes place, it then seriously vitiates the value of inferences drawn from cultural findings after death, when applied to the living lung.

In order to determine the frequency of this occurrence by experimental methods, we have had recourse to rabbits, which have been given small doses of broth cultures of B. prodigiosus just before or after death. Furthermore, we have introduced broth cultures of B. prodigiosus (a half drachm or less) into the mouths of patients who had died in the wards, but before removal of the bodies to the morgue.

The method which we have used for the isolation of B. prodigiosus in the mouths and lungs of rabbits and cadavers was the following: the mouth was scraped with a sterile cotton swab; a suspension was then made in melted agar tubes, and plates with one dilution poured. The mouth plates were made in every case in order to make sure that the patients had received the culture, and thus they served as control for the lung plates. From the lung a considerable amount of expressed juice, obtained by squeezing and crushing a piece of excised lung, was distributed in melted agar tubes and pour-plates made. Both series of plates were then kept at room temperature and observed until the appearance of typical red colonies, or for several weeks. Abundant growths of various organisms were regularly obtained in these plates. In no case have we seen that the development of numerous colonies has inhibited the pigment production.[12]

In nineteen (19) cases, half-drachm or smaller portions of broth cultures of B. prodigiosus were introduced into the mouth by the interne after having pronounced the patients dead. Characteristic red colonies of B. prodigiosus developed on the

[12] A few control experiments were made, to test this point; tubes were inoculated with varying proportions of dysenteric fæces, and broth cultures of B. prodigiosus. These showed that pigment formation by this culture of B. prodigiosus was not inhibited by the excessive growth of other bacteria.

pour-plates from the mouths of these nineteen cases, and in ten cases more or less numerous colonies of B. prodigiosus developed in the lung plates. In other words, in a little over 50 % of the cases evidence was obtained that micro-organisms introduced into the mouths of patients after death enter the lungs. In half of the cases, as seen from Table V, numerous colonies developed on the lung plates.

Since it was impossible to personally control the giving of the cultures after death, we have not included in the table nine cases in which the mouth plates were negative. We, however, believe that of these nine cases some received the cultures, but that the B. prodigiosus failed to develop its characteristic color upon the mouth plates. We have positive knowledge that this occurred in one case (279, not included in table), the cotton swab of the mouth being stained pink. Moreover, as above stated, our B. prodigiosus developed pigment in over-crowded plates; nevertheless, as is well known, pigment formation is subject to a number of disturbing factors which are not readily controlled or perhaps even known.

In support of this view it may be stated that in one case, 175, abundant colonies developed in the lung plates, whereas none appeared in the mouth plates; this case has not been included in the final estimate of the percentages of positive results. For these reasons, we believe that the percentages given are too low for the mouth as well as for the lung plates.

It is certainly a striking observation that likewise in about fifty per cent. (50 %) of our forty-one cases the pneumococcus was isolated. It seems a fair inference from this coincidence that the utmost caution must be observed in the interpretation of the cultural findings of the lung from post-mortem statistics.[13]

[13] In five (5) cases, the prodigiosus culture was received shortly before the exitus lethalis. In one case (82, alcoholic delirium), numerous colonies developed in both the mouth and the lung plates. In two cases (98, 105), the mouth plates alone developed colonies, the lung plates being negative. In case 110, negative results were obtained in the mouth and lung plates; this, however, is readily accounted for by the fact that the culture was administered twenty-four and, again, fourteen hours before exitus. Another of the negative cases (C V), where no colonies developed on the lung plates, is readily enough ac-

We append below the statistics in tabulated form:

TABLE V.

TABLE SHOWING THE CASES IN WHICH B. PRODIGIOSUS, INTRODUCED INTO THE MOUTH POST-MORTEM, WAS RECOVERED FROM THE MOUTHS AND LUNGS.

No.	Mouth.	Lung.	No.	Mouth.	Lung.
166	+	—	214	+++	+
168	+	+++	224	+	—
171	+++	—	233	+++	+
172	+	+	241	+++	—
181	+++	+	258	+++	—
188	+++	++	262	+++	+
199	+	—	278	+++	—
201	+++	+++	281	++	+
205	+	—	288	+++	+++
206	+	—			

Total number of cases..... 19
Mouth positive..... 19
Lung positive..... 7

Our work upon the entrance of B. prodigiosus from the mouths of rabbits to their lungs has been unfortunately confined to two (2) rabbits. One rabbit was given 0.5 c.c. of a broth culture of B. prodigiosus when under ether. The rabbit died twenty minutes later without gasping. The body was then slanted upon a tray with elevated head. (Post-mortem, twenty-nine hours later.) No colonies of B. prodigiosus developed in either mouth or lung plates.

The other rabbit, dying from an intraperitoneal inoculation with the meningococcus, was given 0.5 c.c. of a broth culture, and died slowly several hours later. At autopsy, performed at once after death, pour-plates were made from the mouth and lungs. Numerous colonies developed on the mouth plates; the

counted for by the closure of the glottis from œdema, after a cut-throat wound of the neck. Thus, colonies of prodigiosus were obtained from the lungs in one out of three cases, 33 %, if the above reasons are valid for excluding the two cases (110 and C V). As regards these experiments, however, only negative results are of value, since one cannot make sure that the organisms introduced during life did not reach the lung after death.

lung-plates failed to show red colonies. We abandoned this line of experimentation for the reason that the conditions so obviously differ from those of the human respiratory tract, which almost constantly contains more or less fluid, at least after death.

THE AGGLUTINATING REACTIONS OF THE PNEUMOCOCCUS, STREPTOCOCCUS, AND ALLIED GROUPS OF DIPLOCOCCI.

The impracticabilty of using agglutinating reactions, especially difficult in this group of cocci, led us to forego this aid to species identification. This method, as has been repeatedly shown, when used upon species belonging to the same group, has no differential value, unless very tedious and exact quantitative serum tests are employed. For these reasons, and because of the impossibility of obtaining sera of high valency within the limited time at our disposal, we could not avail ourselves of this method during the greater part of this investigation.

Before giving our conclusions, we deem it best to give the results that we obtained upon the agglutinability of cultures of species belonging to the various groups into which we have divided the various diplococci that we encountered during the progress of our work.

We were able, however, at the end of our work, to make use of the method of obtaining mass cultures for agglutination tests described by Prof. Hiss,[14] which, in his hands, has yielded such brilliant results. With a rabbit immune serum giving a complete agglutination at 1 : 400 with the homologous pneumococcus, which we obtained through the kindness of Prof. Hiss and Dr. Borden, the following tests were made the day after receipt of the serum, which was from a recent bleeding:

Broth cultures were made of species of each of our five (5) groups of cocci, strict attention being paid to the details as given in the method described by Hiss, in the article above referred to. The results of our agglutination tests obtained with the pneumococcus rabbit serum, mentioned above, and with a normal rabbit serum, may thus be briefly described. Two diplococci of Group I were used; the first, 230, a typical pneumococcus, was obtained from a case of suppurative sphenoiditis at autopsy. This pneumococcus did not lend itself

[14] *Journal of Experimental Medicine*, 1905, vii, 223.

to the agglutination test, at least with the mass culture we employed, since the control settled out promptly, and clumps were speedily developed. The second diplococcus, C VII, another typical pneumococcus, was not clumped by the normal rabbit serum, after twenty-four hours, the control showing only a few small, microscopic clumps. With the pneumococcus serum, large flocculi developed in the 1:10 dilution, but the reaction did not become complete, even after twenty-four hours, the fluid still being cloudy. Small flocculi were, however, found in the 1:200, as well as in the 1:1600 dilution, unlike the control.

Only one diplococcus of Group II was tried, namely 71, a typical Streptococcus mucosus, which gave a slight reaction only in the lowest dilution, namely, 1:10. Normal rabbit serum gave no reaction in a similar dilution.

One diplococcus of Group III was tried (199). It gave with the pneumococcus rabbit serum, a positive reaction at 1:100, but no complete reaction was obtained even at 1:10, the fluid becoming only slightly clearer with small clumps on the sides of the tubes. The reaction in the 1:10 and the 1:100 dilutions differed only in the size of the clumps. Normal rabbit serum had no action upon this diplococcus.

Three organisms of Group IV were tried. Culture 49 a gave a slight reaction with pneumococcus serum, in the 1:10 dilution, the clumps being larger than those of the control. After twenty-four hours, all the dilutions and the control, however, became practically clear. Culture 149 was not affected by the pneumococcus or the normal rabbit serum in any dilution. With culture C II the dilutions and the control were practically alike, although possibly there were larger granules in the lowest dilution than in the control.

Two organisms of Group V, typical streptococci, were tested. Culture 214 S did not lend itself to the agglutination test on account of the prompt development of large granules, and the rapid clearing of the fluid in the tubes in the control, as well as in dilutions. With the other streptococcus, 153, larger granules were found, and a quicker settling in the lower dilutions occurred than in the control, which, however, after twenty-four hours, became perfectly clear.

Thus, if one may judge from a single examination, the method of mass cultures or agglutination tests have shown that only in the case of the pneumococcus, C VII, positive agglutinations were obtained, and even with this organism no complete reaction was given in the lowest dilution, namely, 1:10, after twenty-four hours, with the possible exception of the diplococcus 199, of Group III.

It seems to us somewhat forced to draw any conclusions, from the single agglutination test described above, upon the method of using mass cultures, but we believe that the test shows that the diplococci which we have not identified as pneumococci—in other words, all those belonging to other groups than Group

I—have no or very slight agglutinating affinities to the pneumococcus.

Before concluding, it may be well to state that we fully recognize the arbitrariness of our method of classifying the diplococci belonging to the pneumococcus and streptococcus groups.

The experience we have gained in the examination of a large number of cultures convinces us that it is not justifiable to consider all diplococci which ferment inulin, pneumococci. Thus, C II and 149 and 49a are rapid inulin fermenters, but in all other respects they resemble streptococci. It is of interest to note in connection with 49a, that its property of fermenting inulin is a most variable one. Thus, on passage through the first mouse, after its primary isolation from the lungs upon glycerine-agar, it lost its fermenting action upon inulin, only to regain it on further cultivation upon serum water made from the same stock of inulin. Further passages through mice did not affect this function. Again, later cultures of 49a lost their capacity to ferment inulin, but regained it on transplantation, and are now active and rapid fermenters. Cultures C II and 149 have never changed, having always been active fermenters.

We consider that the presence of capsules like the inulin reaction can not be regarded as an infallible guide for the differentiation of these diplococci, especially when the Hiss capsule methods are employed, for the reason that we have encountered streptococci with distinct capsules.

The cocci of what may be called the intermediate groups, III and IV, it may be of interest to state, have been found in the blood during life, and have been recovered from the pial exudate of cases of meningitis. A further study of these interesting diplococci is greatly needed.

CONCLUSIONS.

The following conclusions may be drawn, based upon the result of our researches:

1. Organisms of the pneumococcus or streptococcus group are present in the lungs of practically all cases, whether normal or

showing a variety of lesions; strictly speaking, they were found by us in forty out of forty-two cases, or in 95 % of our series.

2. The pneumococci and the streptococci were obtained in practically similar percentages—that is, in 50 % of the cases.

3. Pneumococci were not obtained more frequently in the small series of patients exposed for some time to hospital atmosphere; our tables show the contrary to obtain. The number of cases examined were, however, insufficient, and the findings may thus be accidental, and hence of no value.

4. Test micro-organisms, namely, small portions—half a drachm or less—of B. prodigiosus, introduced into the human mouth after death, were conveyed to and recovered from the lungs by culture in a little over half of the cases in which this experiment was tried. The test micro-organisms are, we believe, conveyed to the lungs with the fluid which collects in mouths of persons after death, and which in many cases collects just before death. The numerous manipulations entailed in the removal of the body from the wards to the morgue greatly facilitate the entrance of any fluid from the pharynx and buccal cavity into the lungs.

It follows logically, from the results obtained in this experiment, that the cultural findings after death are no guide to the bacterial contents of the lungs during life, and that any deductions made from such findings are unreliable and deceptive. Granted that our explanation be correct, there is every reason to believe that any of the micro-organisms present in the mouths and pharynx and in many cases in the stomach contents may enter the lungs and, if the conditions be suitable, increase in numbers, during the time between death and the examination of the lungs.

5. There exists, perhaps, more frequently than has hitherto been suspected, a series of diplococci, intermediate between the typical pneumococci and streptococci. The diplococci of this type have been found in forty (40) per cent. of our cases.

The differential diagnosis of these atypical diplococci from the pneumococci and streptococci is a difficult one, depending, as it does, upon general cultural characteristics. No single character, such as the presence of capsules or the fermentation of

inulin, virulence, etc., has been found to be a certain criterion. The few agglutinative reactions we have made seem to show that these intermediate diplococci, those of Groups II, III, and IV, have no or only slight agglutinative affinities to the typical pneumococcus. Further tests must, however, be made with the various methods at our disposal before this statement can be accepted as final.

These diplococci are of interest from the fact that they have been found in the blood during life, and in the pial exudate of cases of meningitis, endocarditis, etc.

6. Our studies have thrown no light whatever upon the conditions which determine the onset of lobar pneumonia in apparently healthy persons. Moreover, we have been unable to draw conclusions as to the presence of pneumococci in the lungs during life, or as to the channels by which they gain access thereto.

In concluding, we gladly avail ourselves of the opportunity of acknowledging our indebtedness to Prof. T. Mitchell Prudden for help and advice freely given in the course of this work, and to the various members of the house staff of Bellevue Hospital for their aid and assistance in the pursuit of these researches, especially to Dr. Frank Erdwurm.