A STUDY OF PNEUMOCOCCI: A COMPARISON BETWEEN THE PNEUMOCOCCI FOUND IN THE THROAT SECRETIONS OF HEALTHY PERSONS LIVING IN BOTH CITY AND COUNTRY AND THOSE OBTAINED FROM PNEUMONIC EXUDATES AND DISEASED MUCOUS MEMBRANES.

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The investigations carried on in the Research Laboratory were planned after consultation with the members of the Commission for the Investigation of Acute Respiratory Diseases, of the Health Department of the City of New York, but were otherwise entirely independent of that body. The study of the agglutination characteristics was undertaken by Dr. K. R. Collins, whose report follows this. The investigations are still being carried on and these preliminary reports are made at the suggestion of the Commission, so that all the workers in carrying on further studies might receive help from work already done.

PLAN OF INVESTIGATIONS.

In this study the following points have been considered:

I. The presence of pneumococci (1) in normal sputum, (2) in pneumonic sputum and autopsy material, (3) in the sputum or exudates from pathogenic cases other than pneumonias.

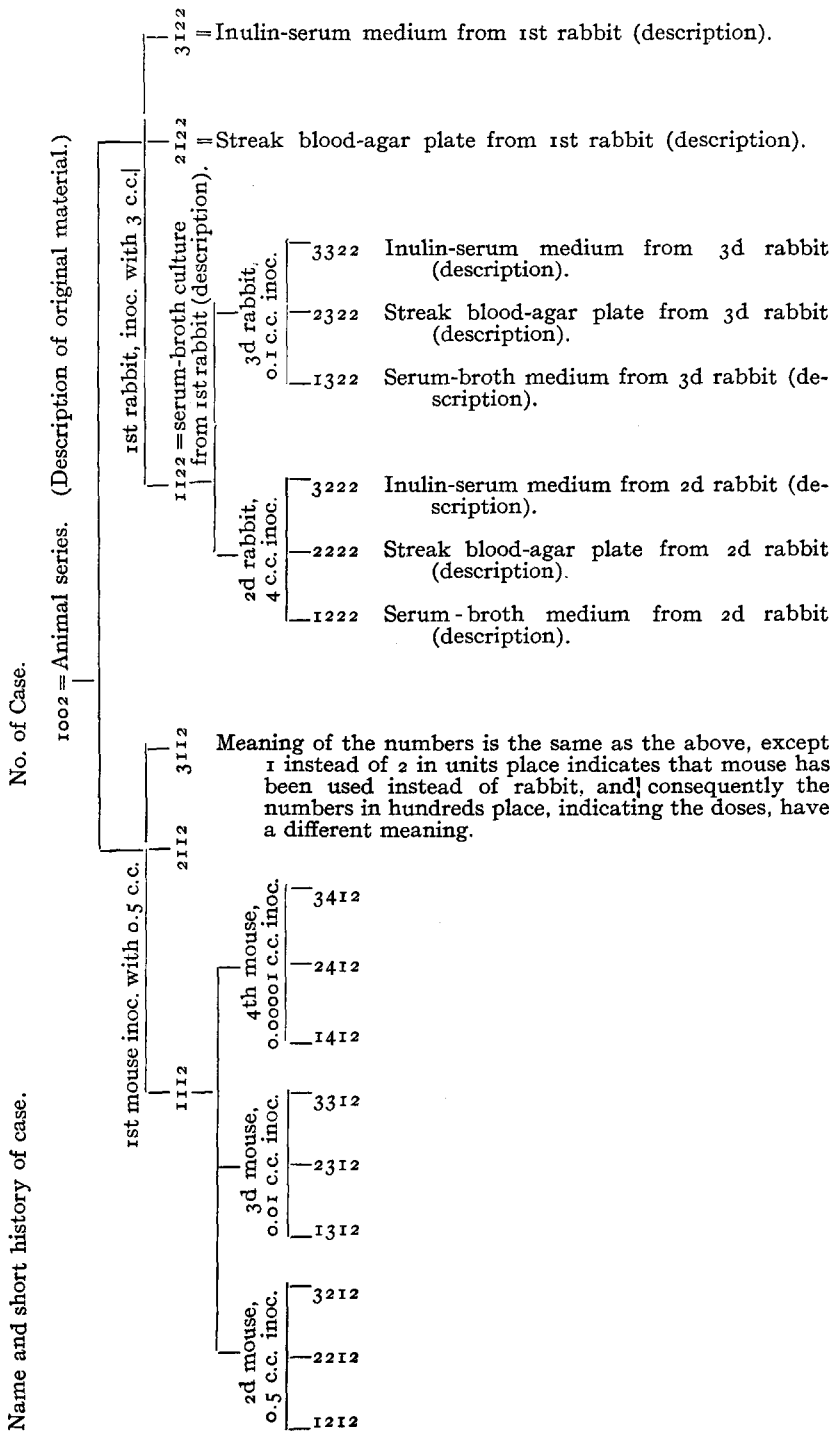
II. The comparison of the strains obtained from the different sources in the following particulars: (1). Morphological and cultural characteristics. (2). Virulence. (3). Serum reactions.

The scheme of the work, which was carried out more or less fully, is tabulated as follows:

TABLE I.

TABLE II.

GENEALOGICAL RECORD.



Meaning of the numbers is the same as the above, except 1 instead of 2 in units place indicates that mouse has been used instead of rabbit, and consequently the numbers in hundreds place, indicating the doses, have a different meaning.

Keeping of Records.—Genealogical tables of each strain have been kept, a modification of the Dewey Library System of numbers being used to indicate the cultures. Thus by referring to these tables one is able quickly to get the principal points in the history of a particular culture from the time of its isolation.

Numbers of four denominations have been used, the units place indicating the series, the tens place the animal used for inoculations, the hundreds place the dose received, and the thousands place the medium employed. Underneath this number the number of culture generations is placed in parenthesis. Table II is one such genealogical table.

In addition to these tables, comment sheets on each strain and tables of comparative morphology and cultural peculiarities have been kept.

THE PRESENCE OF PNEUMOCOCCI.

Two hundred cases have been examined for the presence of the pneumococcus. In the great majority of cases two methods—(*a*) animal inoculation of mass cultures and (*b*) stroke blood-agar plates, as shown in Table I—have been employed in attempts at isolation; in the other cases only one of these two methods has been used. Table III shows the grouping of the cases and the number in which typical and atypical pneumococci have been found.

From this table we see that typical pneumococci have been obtained in a large percentage of normal cases in both city and country. A few pneumococci may have been missed because of occasional contaminations or overgrown cultures or the employment of large rabbits or some other cause. In the majority of cases where no pneumococci were found streptococci were isolated. From a series of autopsies on cases of broncho-pneumonia at the Willard Parker Hospital, and from a series of pertussis sputa from the Foundling Hospital, large numbers of influenza-like organisms were found with smaller numbers of streptococci and occasionally with a few pneumococci. It was very difficult to get rid of these influenza-like organisms, as great numbers passed through the animal inoculated with mass-cultures, and in the plates and serum-broth tubes they grew

abundantly in close association with the pneumococcus. Repeated platings generally had to be made before a pure culture of the pneumococcus could be obtained in these cases.

TABLE III.

SHOWING NUMBER OF CASES STUDIED AND NUMBER IN WHICH PNEUMOCOCCI WERE FOUND.

Groups.	Subdivisions.	Number of Cases.	Pneumococci not Isolated.	Pneumococci Isolated.	
				Atypical.	Typical.
Normal.	Research Laboratory...	3	3		
	Bellevue Students	10	2	1	7
	Saranac Lake	28	15	3	11
	Sea Breeze.....	5	3		2
	Tarrytown.....	1			1
	Foundling Hospital ...	9	2		7
	Briarcliff.....	6	3		3
	Hyde Park	4		4	
	Millbrook.....	7		2	5
	Newburgh	5		1	4
	Babies' Hospital.....	2	2		
Pneumonia.	Lobar-..	53	4	5	45
	Broncho-	21	5	2	14
Colds.		15	5		10
Miscellaneous.	Measles.....	3	1		2
	Scarlet-fever	5	3		2
	Tuberculosis	5	3		2
	Pertussis.....	5	4		1
	Influenza	2	2		
	Pleurisy	1			1
	Typhoid.....	1	1		
	Mastoiditis.....	1			1
	Synovitis	3	1		2
	Meningitis	1			1
	Œdema of Lungs.....	1	1		
	Empyæma.....	3	1		2

COMPARISON OF STRAINS.

Morphological and Cultural Characteristics.—We have divided the pure cultures of pneumococci obtained into two broad groups according to their morphological and cultural characteristics. The first group is composed of typical pneumococci and the second of atypical ones.

By typical pneumococci we mean cocci which (1) under certain more or less constant cultural conditions occur principally in slightly elongated and pointed twos with broader ends

apposed, (2) under similar or other cultural conditions form capsules, (3) when grown in inulin-serum [1] medium produce coagulation, and (4) when grown in poured blood-agar plates produce a distinct green color in and about colonies.

By atypical pneumococci we mean (1) cocci which morphologically and culturally resemble more or less closely the pneumococcus except in their growth in the Hiss inulin medium, which they do not coagulate; (2) cocci which are like streptococci morphologically, but which produce coagulation of the Hiss inulin medium.

Referring to Table III we see that a larger number of atypical strains have been obtained from normal cases, in all of which only sputum was studied, than from pathogenic. This may be due simply to the fact that so many more typical pneumococci were present in the majority of pathogenic cases studied that the atypical ones may have been missed in some of these cases. Atypical pneumococci of the first group, i.e., those which do not coagulate inulin-serum medium, have been found as the majority of colonies and as the only pneumococcus-like organism in the sputum from two cases of pneumonia, and have been accompanied by typical pneumococci in the sputum from three other

[1] The inulin used in the course of the present work in making up Hiss' medium (*Jour. of Exper. Med.*, 1905, vii, 317) was prepared in this laboratory by R. B. Gibson, for at the time we were unable to obtain it from commercial sources. The method employed in the obtaining of this substance follows: Dandelion (taraxacum) roots were soaked in cold tap water until soft, and if coarsely ground the roots were then run through an ordinary hash machine. The material was transferred to a gauze bag, and was washed thoroughly in running cold tap water to remove a portion of the soluble impurities and the finer solid particles which would interfere with subsequent filtration. The washed roots were extracted in boiling water, strained, and filtered. A second immediate extraction followed. The filtrates were united and evaporated over a Bunsen burner to a thin syrup. Alcohol (10–15 %) was added and the mixture was cooled to 0° or below. The inulin separating out on standing was thoroughly washed by decantation with cold alcohol (10–20 %) and then with 95 % alcohol. It was filtered into a suction funnel, washed on the filter with hot alcohol, sucked dry, and finally spread on filter paper in a warm place to remove the alcohol still remaining. The resulting product can be obtained as a fine white powder which gives the ordinary reactions of inulin; solutions of this product do not reduce Fehling's solution. The yield from five pounds of the tubers was about three hundred grams.

cases. So far they have not been found in autopsies following pneumonia. In one autopsy case and in one broncho-pneumonic sputum large numbers of cocci of the second group of atypical pneumococci were found.

It is interesting to note that, when some of the non-coagulating cultures were studied more minutely, various colonies being fished and the resulting cultures being tested for their ability to coagulate serum-inulin media, in the case of one culture one colony out of six produced late coagulation. From this coagulating colony, however, no further colonies were obtained producing coagulation. Among the typical pneumococci all strains vary somewhat with regard to their power to coagulate the Hiss medium, a few producing very late coagulation. When individual colonies were fished from some of these latter strains, it was found that there was a wide variation in the time required for coagulation, an occasional one not coagulating at all. It seems, from these observations, that the non-coagulating, more or less morphologically typical pneumococci are closely related to the typical late coagulators. One of these atypical strains showed typical capsules in the heart's blood of animals and the others showed occasional small capsules.

All of the typical and atypical strains, as well as many strains of streptococci, have produced a green color in and about colonies in poured blood-agar plates, while other streptococci have produced large areas of hæmolysis about colonies and no green color. These results agree in part with those of Schottmüller (*Münchener med. Woch.*, 1903, p. 849), and E. Fränkel (*Münchener med. Woch.*, 1905, p. 548), who divide streptococci into three groups according to their behavior in blood-agar plates: Streptococcus pyogenes producing much hæmolysis, Streptococcus virideus producing green color, and Streptococcus mucosus producing mucous-like material as well as green color. These results differ from those of Rosenow (*Journal of Infectious Diseases*, 1904, I, 280) who states that no streptococcus tried by him produced green color, while all pneumococci did, and he therefore recommends this test in differentiating the two species. From the sputa of a number of cases of broncho-

pneumonia we tried to isolate the pneumococcus by this method, making poured blood-agar plates from different dilutions of the sputum and fishing from the green colonies, and at the same time we used the method of animal inoculation by mass-cultures. In every case by the first method only streptococcus-like organisms were obtained, while by the second typical pneumococci were isolated.

All of the strains of typical pneumococci studied by us may be divided into several distinct morphologic varieties. We call them varieties, because while each strain shows a wide limit of fluctuating variability, certain strains have similar predominating constant characteristics. These varieties are:

1. Small cocci occurring under most cultural conditions in twos and producing small capsules.

2. Large cocci occurring readily in short and medium-length chains and producing large capsules.

3. The so-called Streptococcus mucosus.

The first two varieties are less distinctly bounded than the last which forms a definite morphologic variety. This variety, which has been mentioned only a few times in literature (Schottmüller, *Münchener med. Woch.*, 1903; L. Buerger, *Medical News*, 1904, p. 1117; E. Fränkel, *Münchener med. Woch.*, No. 12, 1905; L. Heim, *Zeit. für Hyg.*, 1905, I, 139), has been classed as a streptococcus, under the name of Streptococcus mucosus by Schottmüller, and Streptococcus mucosus capsulatus by others. It has been isolated by us from eight cases of pneumonia, from two cases of cold, and from two normal individuals, and has been seen in mixed cultures in a number of other cases. Three of the cases of pneumonia were early autopsy cases. In two of these the organism occurred pure and in large numbers (two hundred colonies were fished in one case and the resulting cultures were all similar); in the third case it was accompanied by a smaller number of the first variety of typical pnuemococci. In one pneumonic sputum and in one normal individual the first variety of typical pneumococci also accompanied it, while in all the other cases it was the only pneumococcus-like organism isolated. It has thus been found by us more frequently in

cases of pneumonia than in other cases. We have classed it among the typical pneumococci for the following reasons:

1. On serum-free culture media after the first two or three culture generations it produces no mucous-like material and shows no capsule or chain formation, but appears like a typical pneumococcus.

2. It readily coagulates the Hiss inulin medium.

3. It shows very distinct capsules in serum media and in the blood of inoculated animals.

4. It has been found pure and in large numbers in two cases of typical lobar pneumonia.

5. The results obtained from absorption experiments (see the Collins report) indicate a close relationship between it and certain typical pneumococci of the second variety, while no relationship is shown between it and the strains of typical streptococci tested.

It has been classed with the streptococci heretofore because of its ability readily to produce rounded forms and short chains. According to our descriptions of typical and atypical pneumococci it might be classed by many with the latter, but considering its ability under certain conditions to show typical pneumococcus forms we prefer to class it with the former, and make it a distinct variety. With regard to nomenclature, it should be called, according to the classification followed, Streptococcus lanceolatus, var. mucosus (the classification of Lehmann and Neumann, which we prefer), or Diplococcus lanceolatus, var. mucosus (the classification of other authors); we have given it the trivial name, Pneumococcus mucosus.

By refering to the section on serum reactions below and to Dr. Collins's report on the agglutination of the pneumococcus, it will be found that all the strains of this variety isolated by us show a specific similarity in these reactions.

A certain number of cultures from both normal and abnormal cases, which showed the characteristics of typical pneumococci immediately after isolation, have later dropped some of these characteristics and become more like streptococci. They appear principally in chains and no longer coagulate the inulin-serum medium. Whether some of these cultures were mixed in the

beginning with a streptococcus-like organism growing in intimate connection with the pneumococcus, as the influenza bacillus does, and finally outgrowing it, or whether they are all mutating varieties, is still a question. With such a mass of cultures it was impossible to follow each closely, to make plates, and to study colonies of each new culture generation, but, judging from the few apparently changing strains which have been more minutely studied, it would seem as if some of these cultures were really changing by mutation. None of them have become permanently typical streptococci—that is, they show more or less irregularity in chain production, sometimes produce elongated and pointed twos and always green color in blood-agar plates, but they seem gradually to lose their power to coagulate inulin-serum medium. These observations in regard to mutating varieties indicate a close relationship between certain pneumococci and streptococci, a relationship which previous investigators have noted.

All strains of pneumococci tried coagulated, usually within forty-eight hours, serum media containing dextrose, lactose, or saccharose, as do also certain strains of streptococci. With mannit different strains of pneumococci act differently. Out of one hundred strains tested, twenty-nine did not coagulate mannit-serum medium after fourteen days. Among the seventy-one coagulators, sixteen coagulated in twenty-four hours, seventeen in forty-eight hours, one on the third day, five on the fifth day, and the rest between the fifth and fourteenth days. With the exception of the mucosus variety, there seems to be no relation between this coagulation and the varieties or groups of pneumococci. All of the Pneumococcus mucosus strains tested coagulated the mannit medium within two days. Certain atypical strains which did not coagulate the inulin readily coagulated the mannit medium, while the few definite streptococci tried did not coagulate either. The plate growths from these non-coagulating cultures all showed practically as many colonies as those from the coagulating ones.

Virulence.—The virulence of the different strains of pneumococci for lower animals depends in great measure upon the method of isolation used. If the plate method be employed, fishing individual colonies, the majority of pure cultures ob-

tained will be distinctly less virulent than those isolated by the mass-culture method. The mass-culture method consists in inoculating a mass of sputum or material to be tested into serum-broth (previously tested for ability to give abundant growth of pneumococci), placing at 36° C. for twenty-four hours, and inoculating a certain amount of the resulting culture subcutaneously into the animal chosen. The culture isolated from the heart's blood of the animal at autopsy is then tested for virulence in the same species of animal.

We have used both rabbits and white mice for the inoculations, but in the great majority of cases the former animals only. Young rabbits, weighing from 800 to 1000 grams, and young adult mice have been chosen.

By testing the virulence of strains isolated by the mass-culture method, it has been shown that the percentage of virulent strains of pneumococci isolated from cases of pneumonia is higher than the percentage of those isolated from normal cases (see Table IV).

TABLE IV.

PERCENTAGE OF VIRULENT STRAINS.

Amount Inoculated.	Pneumonia Cases.	Healthy Individuals.
4.0 c.c.	87 %	69 %
0.1 c.c.	51 %	31 %

Most of the strains isolated from the cases of broncho-pneumonia which are included with the cases of pneumonia are not very virulent, while most of the strains from the colds which have not been noted here are virulent. Among the normal individuals the largest percentage of virulent pneumococci came from the Foundling Hospital children, the next from the Bellevue students, the next from the country around New York, and the smallest from Saranac Lake. Too much weight should not be attached to this summary, because of the comparatively small number of cases examined.

Normal No. 40 in contact with Pneumonia No. 36 were of equally extreme virulence for both mice and rabbits. All of the

Pneumococcus mucosus strains tested have been with one exception extremely virulent for mice and decidedly less so for rabbits.

Retention of Virulence.—Grown on artificial media, all of the virulent strains are losing their virulence although those transplanted on media containing blood from the species of animal used for the testing remained more virulent longer, for that species than for the other species of test animal chosen. No. 36, however, one of the most virulent strains tested, remained virulent for a long time for both rabbits and mice when grown on rabbit blood-agar. It seems now gradually to be losing its virulence for both animals. When grown in Bolduan's calcium-broth medium (*New York Med. Jour.*, May, 1905), cultures of pneumococcus remain alive and retain their virulence as long as when grown in serum-broth according to the few tests made; therefore, as this medium generally allows an abundant growth, it is an excellent one to use when for any reason the use of serum is undesirable.

Agglutination reactions are described in a separate report by Dr. Collins.

SERUM REACTIONS.

Specific Protective Substances.—According to Neufeld and Rimpau (*Deutsche med. Woch.*, 1904, p. 1458), the serum of rabbits inoculated with pneumococcus cultures becomes speedily protective for white mice. They claim to have obtained after the second inoculation of large doses of pneumococcus bodies, the first killed by heat and the second living, a serum which was highly protective for mice. They claim that this serum has no lytic properties for the pneumococcus, but that the specific protective substance is a bacteriotropic substance uniting with the bacteria and preparing them for ingestion by the leucocytes, and that when this serum is added to a mixture of bacteria and normal leucocytes in vitro more phagocytosis is produced than when normal serum is used. So far Neufeld's bacteriotropic substance agrees with Wright's (*Proceedings of the Royal Soc. of London*, 1903, LXXII, 337) opsonic substance, except that Neufeld claims that his substance is not destroyed by low heat, while Wright says that his is. Therefore Neufeld states that his bacteriotropic substance is not the same as Wright's opsonic substance.

Very little as yet has been done by us in attempting to raise in animals specific protective substances for the pneumococcus or in studying the properties of such substances. In the beginning we followed Neufeld's method, inoculating large doses of surface cultures of pneumococci into rabbits. The first cultures were subjected to from 60° to 65° C. for from fifteen to thirty minutes and the subsequent cultures were living. There is no doubt that a preliminary large dose of a dead culture could be followed by a larger dose of a living culture without causing death than if a small preliminary dose had been used, but the serum of such rabbits showed no protective action in mice with any of the strains of pneumococcus tested. Only a few tests were made, however, so no definite conclusion can be drawn. The phagocytic power in vitro seemed to be slightly increased for some of the strains, each by its own serum.

It was found that the opsonic power of normal rabbit, sheep, and especially of normal horse serum is very great for some strains of pneumococci, less so for others, and very slight for others. All of the strains of Pneumococcus mucosus tested belong to this last group. Since rabbits proved unsatisfactory, it was decided to experiment with sheep. Two sheep were chosen, one of which was inoculated with one of the first variety of pneumococcus and the other with a strain of Pneumococcus mucosus. The sheep have received eleven inoculations and have been bled twelve times. (See Table V.)

The serum from each bleeding was tested in vitro for its opsonic or bacteriotropic power on a number of strains of pneumococci, and from a few of the bleedings it was tested in addition for its protective power in white mice. In testing the opsonic power of the serum in vitro the following technic was used: To 0.5 c.c of serum, undiluted or diluted, in a short wide test tube, were added 0.5 c.c. of a thick suspension of washed normal leucocytes and 0.5 c.c. of the dilution of bacteria. The washings and dilutions were made with 0.8 % of sodium chloride solution. The mixtures were kept at 36° C. and smears made at stated times. The leucocytes almost immediately form a thin layer about the sides and bottom of the test tube and a well spread smear

containing large numbers of leucocytes is made by scraping from this layer with a flatly coiled platinum loop and spreading quickly on a clean glass slide. The smears were fixed in methyl alcohol and stained with eosin and methylene blue. Normal leucocytes from rabbits, guinea-pigs, sheep, and horses have been used, and so far our results have agreed with those of all other

TABLE V.

INOCULATIONS AND BLEEDINGS OF SHEEP.

Amount Inoculated.	Date of Inoculation.	Date of Bleeding.
20 c.c. of 24-hr. broth cult. centrifugalized and exposed to 60° C. for 20 min.	March 3	—
27 c.c. of 24-hr. broth cult. centrifugalized and exposed to 60° C. for 20 min.	March 10	March 15
28 c.c. of 24-hr. broth cult. centrifugalized and exposed to 60° C. for 10 min.	" 17	" 24
32 c.c. of 24-hr. calcium-broth cult. centrifugalized.	" 29	April 2
40 c.c. of 24-hr. calcium-broth cult., 30 c.c. centrifugalized and 10 c.c. non-centrifugalized.	April 8	—
50 c.c. of 24-hr. calcium-broth cult., 30 c.c. centrifugalized and 20 c.c. non-centrifugalized.	" 19	April 27
60 c.c. of 24-hr. calcium-broth cult., 30 c.c. centrifugalized and 30 c.c. non-centrifugalized.	" 28	May 4
70 c.c. of 24-hr. calcium-broth cult., 30 c.c. centrifugalized and 40 c.c. non-centrifugalized.	May 5	" 10
80 c.c. of 24-hr. glucose-calcium-broth cult., 30 c.c. centrifugalized and 50 c.c. non-centrifugalized.	" 15	" 24
85 c.c. of 24-hr. glucose-calcium-broth cult., 30 c.c. centrifugalized and 55 c.c. non-centrifugalized.	" 27	June 5
50 c.c. of 24-hr. glucose-calcium-broth cult., +10 slant blood-agar cult.	June 8	" 15
	—	" 21

observers in regard to the indifferent action of leucocytes from different species of animals. According to our experiments, some species of leucocytes need more careful washing than others, probably because of the greater opsonic power of the corresponding normal serum. For example, horse leucocytes must be most carefully washed in order to keep the controls from showing phagocytosis. We have used horse leucocytes for many of the experiments because of our ability to obtain them easily and quickly in large quantities. The horse is bled just before the leucocytes are to be used and the blood is collected aseptically in flasks, with one tenth its volume of a 10 % solution of sodium

citrate in normal salt solution. After mixing, the blood is allowed to stand, and within ten minutes the red blood cells have settled, leaving the plasma, containing many leucocytes, above. This is drawn off, centrifugalized, and the leucocytes are washed carefully four times; each time fresh sterile plugs are used for the tubes. In this way it is easy to obtain a large amount of a very thick suspension of actively motile polynuclear leucocytes. Of such a suspension 0.5 c.c. added to the mixture of 0.5 c.c. each of 0.8 % salt solution and the required dilution of bacteria has been used as one control, and a similar mixture with normal serum in the place of the 0.8 % salt solution as another.

The dilutions of the bacteria were prepared as follows: A twenty-four-hour calcium-broth culture made from a twenty-four-hour blood agar-slant culture of the stock culture (the blood-agar was made from rabbit blood and kept in the thermostat at 36° C. for two days before using) was centrifugalized and enough 0.8 % salt solution added to the bacteria to make a suspension of about 2,000,000,000 bacteria to the cubic centimeter.

In estimating the phagocytic action by this method, it has been found that a large number of polynuclear leucocytes must be counted, as phagocytosis seems to occur irregularly, a group of polynuclear leucocytes each one loaded with bacteria filling one field, and a group containing no organisms the next.

The mixtures were examined in the beginning, at the end of one quarter, one half, two, three, five, and twenty-four hours. It was found that the difference between the serum controls or heated serum and the specific serum was more marked after fifteen to thirty minutes than at the height of phagocytosis, which occurred in from two to three hours. At the latter time the differences, if any, were very slight. The specific serum thus seemed to allow the phagocytosis to occur more quickly.

The difference between the opsonic power in vitro of the normal serum and the specific serum, however, has so far been slight. This slight increase of opsonic power of the specific serum was apparent after the second bleeding and continued up to and including the eighth bleeding, but the serum from the next two bleedings (ninth and tenth) showed no definite difference in

phagocytosis between normal serum controls and specific serum. The serum from the ninth bleeding, however, showed a protective power for mice similar to that of the serum from the eighth bleeding, and the serum from the tenth bleeding prolonged life. As the control animals in these experiments all died, the absolute protective power of these sera is not known. From these data all that can be said is that while the phagocytic power in vitro of a certain specific serum seemed no greater than that of the control serum, yet the former possessed marked protective power in mice. One of the heterologous strains (Pn. 4) showed clumping and marked phagocytosis with Sheep Serum II (inoculated with Pneumococcus mucosus), while with Sheep Serum I (inoculated with Pn. 36) it showed no clumping and less phagocytosis, and yet mice were protected from it by this latter serum. All of the Pneumococcus mucosus strains showed very slight phagocytosis in any serum, and yet with Sheep Serum II mice were protected from all of the strains with but one exception.

It seems from these observations that the degree of phagocytosis in vitro with some sera at least is not an indication of the degree of protective power in mice.

In regard to the influence of heat upon the phagocytic power of these sera, the following results have been obtained: 60° C. for a half hour has slight deleterious effect, 65° for twenty minutes has more, and 60° for one hour has a marked effect.

Poured blood-agar plates after two hours at 36° C. show a decrease in the number of colonies with all the strains which agglutinated, but the decrease is no greater than could probably be accounted for by the agglutination.

SUMMARY AND CONCLUSIONS.

1. Typical pneumococci were present during the winter months in the throat secretions of a large percentage of healthy individuals in city and country.

2. A higher percentage of atypical strains of pneumococci have been obtained from healthy persons than from those suffering from pneumonia. In the latter cases the atypical strains may have been overlooked, because of the larger number of typical

pneumococci present. Many of the atypical strains seem to be closely related to the streptococci.

3. The so-called Streptococcus mucosus Schottmüller, which has hitherto been classed with the distinct streptococci, is placed as a definite variety among the pneumococci, and it is recommended that the name be changed to Streptococcus lanceolatus, var. mucosus.

4. A lower percentage of strains of pneumococci virulent for rabbits in the doses used has been obtained from normal cases by rabbit inoculations of mass cultures than from cases of pneumonia by the same method.

5. Since the virulence of pneumococci may be rapidly increased for a susceptible species of experimental animal by successive passage, and since pneumococci obtained from most pneumonias are more virulent for experimental animals than are those obtained from healthy individuals, therefore the virulence of pneumococci from cases of human infection is probably increased for human beings; hence cases of pneumonia should be considered to a certain degree as contagious and, since the virulence of the pneumococcus may be quickly increased and since the organism is very prevalent in normal sputum, all possible measures should be taken to restrict public expectoration.

6. By repeated inoculations into sheep of a pneumococcus strain, a specific protective power of this serum for mice is developed against the homologous strain and against certain other strains, one morphological variety (Streptococcus lanceolatus, var. mucosus) being thus clearly differentiated from other strains.

7. Coincident with this production of protective power, a slight specific increase of the sheep serum in phagocytic power in vitro has been observed with some strains of pneumococci, all strains of Streptococcus lanceolatus, var. mucosus, acting similarly with the serum produced by the inoculation of one strain; the strains of some other varieties, however, have shown no definite relationship between the phagocytic power and the protective power of the serum.

NOTE.—The protocols of cases and experiments will be published later in the Reports of the Health Department of New York City.