

REPORT OF THE DIRECTOR OF THE HOSPITAL  
OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

January 1919

To the Board of Scientific Directors of  
The Rockefeller Institute for Medical Research.

Gentlemen:

During the past quarter the same conditions that have restricted the research activities of the Hospital during the past year have continued to be operative. In addition to the absence of many of the staff, Dr. Van Slyke has been ill, and consequently there has been little work accomplished in the chemical laboratory, aside from routine work and teaching. Dr. Cohn is still abroad so that the work in his department has been practically at a standstill. The interne staff has consisted entirely of army officers detailed to the Hospital. These men are on temporary duty, subject at any time to orders sending them elsewhere. Most of them have had little or no training in research methods. Consequently it has been practically impossible for them to engage in investigation. On account of the character of the interne staff, it has been necessary for the Director to give much of his time to the actual diagnosis and treatment of patients in the wards.

The work of training members of the army medical corps has continued during the quarter and has occupied a considerable amount of the time of the staff. This work, as mentioned in the previous reports, has consisted of four special courses:

I.-A course in bacteriological methods given for medical officers in the Institute Laboratories. The instruction of the men in the methods of studying the bacteriology of acute infections has been given by Dr. Avery and Dr. Dochez, assisted by Dr. Stillman, and this has required a considerable amount of their time.

II.-A course in chemical methods for members of the Sanitary Corps has been given in the chemical laboratory of the Hospital.

III.-A course designed to teach women the bacteriological methods employed in the etiologic diagnosis of acute respiratory diseases has been given during the past quarter in the Hospital. This course has been under Dr. Avery and Dr. Stillman.

IV.-Training medical officers in clinical methods. These officers have also acted as internes.

All these courses have now ended and it is not likely that there will be any further need for this kind of activity at present. A few of the army medical officers ordered to the Hospital for clinical study still remain, but as soon as they are replaced by civilians they will undoubtedly be sent to duty elsewhere.

In spite of the conditions during the war, it has been possible to maintain the essential character of the Hospital, and with the coming of peace, it seems likely that a permanent staff can soon be assembled and that the work of the Hospital can proceed in orderly fashion once more. Nevertheless, much reorganization will be necessary, and it seems advisable not to attempt to obtain a full staff at once, nor to attempt to resume all the lines of work in progress at the opening of the war. So much depends upon obtaining exactly the right kind of men to assist in the work that it seems advisable to resume the work and to replace the staff

gradually as the problems develop.

#### Study of Acute Respiratory Diseases.

The previous report was written very soon after the epidemic of influenza had made its appearance in the army camps and when it was beginning in New York. Since then, this disease has prevailed to a very great extent in New York. We have, therefore, been compelled by circumstances to extend our study of acute lobar pneumonia to the study of influenza. Indeed, the character of the acute pulmonary infections in New York has so changed during the past year that now typical lobar pneumonia in our experience has been largely replaced by pulmonary infections complicating influenza and by atypical inflammations of the lung.

We have admitted to the Hospital a considerable number of persons suffering from influenza in whom no extensive pulmonary lesions have developed. These were largely members of the staff and working force of the Institute. These patients offered excellent material for study of the milder uncomplicated cases of influenza. As far as possible, however, the admission of patients from the outside was confined to those in whom pulmonary lesions were present or were suspected at the time of admission. For this reason, the disease as seen in the Hospital was of somewhat more severe character than the disease as it existed in the city at large. This is evident from the high percentage of pneumonia and high mortality of the disease in the Hospital.

The observation of the earliest cases made it evident that the etiology of the disease was uncertain. With the depleted staff and other work demanding attention it was obvious that it was impossible for us to investigate the etiology from all standpoints. It therefore seemed advisable for the Hospital staff to confine its attention

mainly to the study of the rôle of the influenza bacillus in the epidemic and to the investigation of the other microorganisms concerned in the pulmonary complications, leaving to members of the Laboratory Staff the search for an unknown virus. Dr. Amoss and Dr. Olitsky, therefore, undertook the latter problem, obtaining material for this investigation from the cases treated in the Hospital. Their results are given in the report of the Director of the Laboratories.

The attempt has been made in the Hospital to study with as great care as possible the predominating organisms present in the throats and sputum of all the cases of influenza and pneumonia during life, and to study the bacteria present in the lungs at autopsy. It has been the intention to make this study with as great accuracy as possible on the limited number of patients coming under our observation. This has involved a larger amount of work than might seem necessary at first sight. There were, at first, many technical difficulties, especially in the isolation of *B. influenzae*. We have thought it necessary in all cases, in order that we might be certain that we were really dealing with this organism, that it be obtained in every case in pure culture and that the organism so obtained be tested by all known methods. Moreover, in order to be certain that influenza bacilli were not being overlooked, it was necessary in many cases to make repeated cultures. With greater experience and especially with the improvement in method of isolation devised by Dr. Avery, of which mention will be made later, the difficulties in the study have been much diminished.

The following table shows the frequency of occurrence of influenza bacilli and other predominating organisms in the cases of influenza and pneumonia studied in the Hospital during the past quarter.

	Total number	B. influenzae present %	Other Predominating Organisms								Died	Per cent
			Pneumococci				Staphylococci	Non-hemolytic streptococci	Hemolytic streptococci	B.Friedländer		
			I	II	III	IV						
Uncomplicated influenza	49	83		2	2	17	1	2	2		0	0
Influenza with pneumonia	43	93	1		3	27	3	1			12	28
Atypical pneumonia influenza (?)	8	100			1	4	2			1	2	33
Lobar pneumonia	<u>18</u> 118	55	<u>3</u> 4	<u>2</u> 4	<u>6</u> 12	<u>7</u> 55	<u>—</u> 6	<u>—</u> 3	<u>—</u> 2	<u>—</u> 1	<u>7</u> 21	<u>35</u> 18%

In most cases cultures were made from the throat and sputum, and in the cases coming to autopsy, from the various organs. The cultures were made by various methods, by mouse inoculation, culture on blood agar plates and on oleate agar. The latter method proved by far the most successful. The most striking fact brought out is the frequent occurrence of *B. influenzae* in the influenza and broncho-pneumonia cases. In the cases of lobar pneumonia, *B. influenzae* has been encountered less often than in the influenza cases, and with more nearly the same frequency as in normal throats during the same period. Hemolytic streptococci have been found very seldom, in only two throats. An interesting fact is that in six cases staphylococci were present in large numbers, in five of the pneumonia cases they were the predominating organism and seemed to be responsible for the complication.

The clinical features of the cases have not yet been carefully analysed. No striking clinical differences have been present in the cases of influenza here from those described in the cases occurring elsewhere. No extensive study of the blood changes have been undertaken. Fairly frequent blood counts have been made in a routine manner on all cases. Most of the cases have shown the leucopenia which has been generally observed.

Distribution of *B. influenzae* in the Mouths of Normal Persons  
and of Convalescents.

On account of the great prevalence of *B. influenzae* in the cases of pneumonia and influenza occurring in the Hospital it has seemed of importance to determine the frequency of their occurrence in normal mouths during the same period. It was planned to make a complete survey of the mouth flora of the workers and staff of the Institute, and this work has been undertaken by Dr. Stillman, assisted by Miss Pritchett. Some

time was lost in developing a proper technique for isolating B. influenzae but the work is now progressing at a fairly rapid rate. Unfortunately Miss Pritchett must leave to undertake her new duties at Baltimore but her work will be continued by Miss Winchell, and it is hoped that by making fairly frequent cultures, curves can be made of the occurrence of carriers of B. influenzae in the various departments during the coming months. It is realized that this Institute is not ideal for such a survey as the population is somewhat shifting, and since most of the persons working here have wide contacts outside the institution. Nevertheless, the advantages of making such a study where the facilities for work are so satisfactory, and where the residents can be constantly under the observation of the investigators, seem to outweigh the disadvantages, and it is hoped that such a survey may throw some light on the distribution of influenza and coryza, and possibly on the etiology of the disease.

Up to the present time, a study has been made of 231 individuals. In most instances cultures have been made only once, and the present figures indicate roughly the conditions present in this Institute during the latter part of November and December.

Of the 231 persons, 54 had suffered from influenza during the past three months and should therefore be considered convalescents, 177 gave no history of having had this disease. From the sputum or throats of 41 per cent of these normal individuals, influenza bacilli have been isolated. Of the 54 persons in whom cultures were made during convalescence from influenza, 46 per cent showed the presence of B. influenzae in the throat.

Almost one half the persons in the Institute who have been studied during the past months, therefore, have carried B. influenzae. Whether this wide distribution is constant or is only associated

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with the presence of the epidemic, only further studies will show. The latest studies indicate, however, that the incidence of this organism in normal throats at present is much less than it was a couple of months ago. Moreover, the distribution of the carriers of this organism has been interesting. For instance, in the preparation room, where there were two convalescent carriers, all of the eight persons working there carried this organism. On the other hand, in the chemical laboratory, none of the workers were found to harbor *B. influenzae*. Strangely enough, very few carriers have been found among the nurses in the Hospital.

It is obvious that no conclusions should be drawn from this study at the present time. The line of investigation, however, seems promising.

#### Study of Biological Properties of *B. influenzae*.

Dr. Dochez and Dr. Avery have been engaged in a study of the properties of the influenza bacilli isolated in this Hospital. One fact observed which is of much practical importance is that the growth of this organism is enhanced by the addition to media of certain soaps of the unsaturated fatty acids. This favoring action to growth seems exerted generally in the case of Gram negative organisms and the opposite effect is observed in the case of Gram positive organisms, especially streptococcus and pneumococcus. Use has been made of this observation in the production of a selective medium for *B. influenzae*. The medium is prepared by the addition of sodium oleate to 2 per cent meat infusion agar in such amount as to make the concentration of oleate 1 to 1000. The initial hydrogen ion concentration of the agar should represent a pH of 7.3 to 7.5. To 100 parts of the agar, while hot, there is added 1 cc. of a suspension of rabbit's corpuscles. Because of the more luxuriant growth upon this medium, the

colonies of *B. influenzae* appear larger and less translucent than upon ordinary blood agar, and in later stages of development are not infrequently nucleated. Upon this medium, the Gram negative cocci of the catarrhalis group, staphylococci, and occasionally diphtheroid bacilli, grow, while pneumococci and streptococci of the hemolytic and viridans group fail to develop. The use of this medium has very greatly facilitated the study of the occurrence and distribution of *B. influenzae*. A further observation has been made that Gentian violet in dilution of 1:100,000 - 1:50,000 still further restrains the growth of Gram positive organisms, eliminating staphylococcus, without materially influencing the growth of *B. influenzae*. It has also been noted that, contrary to the general belief, the influenza bacillus lives for as long a period as six weeks in blood broth without transfer in the incubator at 37° C., at room temperature, and at 8° C.

It has been found possible to raise the virulence of *B. influenzae* by passage through rats and mice, so that 1/50 cc. of a 24 hour blood broth culture will kill a rat and .001 cc. prove fatal for white mice. Under these conditions the organisms are found in great numbers in the hearts' blood of these animals.

Fermentation Reactions. A preliminary study of the fermentative reactions of *B. influenzae* indicates that this organism forms little or no acid from various test substances used. Because of this fact use cannot be made of carbohydrate media containing the usual acid indicators. Use is therefore being made of the method for determining the hydrogen ion concentration of cultures of this organism.

#### Toxin Production.

A study has been undertaken to determine whether or not *B. influenzae* by growth in fluid media produces a soluble toxin of

any considerable degree of potency. Such studies up to the present have in the main been negative. It has, however, been possible to produce in fluid media a substance of sufficient toxicity to kill white rats in doses of 0.5 cc. of the bacteria-free fluid.

In the course of bacteriologic study of patients in the wards suffering from influenza, it was found that in addition to the typical B. influenzae, a different Gram negative hemoglobinophilic bacillus was present. This differs essentially from the influenza bacillus in the following characters: greater uniformity in staining property, marked alkaline fermentation of milk with subsequent peptonization and active hemolysis of blood cells. A further study of this organism is being made.

Dr. Lyon has undertaken the artificial production of immunity in rabbits to virulent cultures of B. influenzae. This work has not yet progressed far enough for a report of the results to be made.

#### Further Study of Atypical Type II Pneumococci.

It has been found that a considerable number of pneumococci isolated from cases of pneumonia, and also from normal mouths, react atypically with Type II serum. Some study has been made of these organisms by Dr. Avery, the results of which have been previously reported, and which have been published. From a study of ten of these strains Dr. Avery was able to classify them into three types, II a, II b, and II x. By means of agglutination, absorption and protection experiments, the members of sub-types II a and II b were found to possess immunity reactions identical with all other strains of the homologous types. Sub-type II x, however, consists of a heterogeneous series of independent strains which showed neither cross-agglutination nor cross-protection.

Dr. Stillman has collected 204 strains of these

atypical type II pneumococci and has made a complete study with the purpose of elaborating further their classification on the basis of their specific relationships. The source of the strains studied was as follows:

Lobar pneumonia	77
Post-operative pneumonia	2
Meningitis	1
Guinea pig pneumonia	5
Normal mouths	100
Convalescent Type I pneumonia	6
"        "    II        "	3
"        "    III        "	1
Dust	<u>6</u>
Total	204

In classifying these strains agglutination and absorption reactions have been employed. On the basis of specific agglutination in monovalent rabbit serum, the 204 strains have been classified into twelve distinct groups. Only two strains showed cross-agglutination in the immune serum of a heterologous group, and these reactions were shown to be due to the presence of minor agglutinins for one group. Organisms belonging to certain sub-groups, namely, II a and II h, have been encountered much more frequently in relation to disease and it is probable that these have a greater pathogenicity. Organisms of these groups occur rarely in normal mouths. Just the opposite is the case with organisms of certain other groups such as groups II b, II c, II f, and II m. Organisms belonging in these groups are much more frequently encountered in normal mouths than in association with disease.

### Biologic Classification of Streptococcus Hemolyticus.

In my previous report mention was made of the study being carried out by Dr. Dochez and Dr. Avery on hemolytic streptococci, and it was then stated that evidence had been obtained that the various strains of hemolytic streptococci differ in their immunologic reactions, and that these organisms may be grouped on the basis of common characters, just as is the case with pneumococci and meningococci. While the relations between the various types of streptococci are more complex than is the case with pneumococci, and while there have been considerable technical difficulties to be overcome in working with streptococci due to (1) the difficulty in rendering them virulent for mice, (2) their tendency to spontaneous agglutination, (3) difficulty in immunizing animals, nevertheless, the work has progressed favorably, a considerable number of strains have now been thoroughly studied, and the results of the investigation will soon be ready for publication.

### Treatment of Syphilis with A 189

#### Dr. Stillman

The quarter opened on October 1, 1918, with fifty-four syphilitic patients in the Hospital. All of them with two exceptions were soldiers or sailors. Since October 1, thirteen patients have been admitted to the Hospital, the number of admissions being curtailed on account of the recent epidemic of influenza and the need of hospital room for these cases.

Fifty-three patients have been discharged from the Hospital during this period, so that seventeen syphilitic patients were in the Hospital on the first of January 1919. Of the fifty-three patients discharged, forty-five left as improved, that is with a negative Wassermann

reaction both in the blood and in the spinal fluid. Four were admitted to the Hospital with a diagnosis of syphilis but were found to be not syphilitic. Two were transferred to other hospitals on account of complicating infections, and the only syphilitic patient who was discharged with a positive Wassermann was a congenital syphilitic who is to return later for further treatment.

During the month of October there occurred three severe cases of dermatosis directly attributable to treatment with A 189. Toward the end of October, 1918, one of these cases died of acute exfoliative dermatitis. There also occurred two cases of severe acute conjunctivitis. The occurrence of these complications made it seem incumbent to revise somewhat the mode of application of the drug, so that since October 20th the drug has been given at two week intervals in doses of seven milligrams per kilogram of body weight. It has been dissolved in  $2\frac{1}{2}$  molecules of sodium hydrate and 750 parts of saline solution.

The analysis of results presented in the October report indicated that variations in alkalinity and dilution within the limits used have no effect on the reactions occurring in the administration of A 189; and therefore the fact that during this quarter we have had no severe reactions may be attributed to a lengthened interval between doses. The lengthened interval between doses may be accountable also for the two following conditions which have occurred in our recent treatment:

1. In two cases mucous patches have recurred directly under our observation, from each of which Treponemata pallida have been isolated.
2. Two cases have developed both clinical and laboratory findings of central nervous syphilis under treatment, but were definitely known not to have either before the institution of treatment.

It appears possible, therefore, that the diminution

in toxic effects, gained by administering A 189 at approximately half the previous rate, may be attained at the cost of lowered therapeutic efficiency.

#### Chemical Laboratory.

Owing to Dr. Van Slyke's illness and the reduced staff, the research activities in the chemical laboratory have been markedly diminished during the past quarter. Dr. Cullen has been continuously employed at the War Demonstration Hospital. It is hoped that he will soon be free to resume his work here. A considerable amount of the space and facilities of the chemical laboratories have been used during the past quarter by the men of the Sanitary Corps taking the course in clinical laboratory methods under the direction of Capt. Robinson.

Miss Hiller has continued the work on which she was engaged with Dr. Van Slyke when we was compelled to leave. This problem consisted in ascertaining the optimum nitrogenous nutrient for pneumococci. Neither intact proteins nor the amino acids resulting from their complete hydrolysis appear to be as good for pneumococcus media as some of the products of partial hydrolysis termed indefinitely "peptones". The object of Miss Hiller's work is to ascertain how completely it is desirable to digest the proteim pabulum in order to get it into optimum condition for the bacteria to grow on. The preliminary work has been a study of the digestion of casein with trypsin under accurately reproducible conditions, the curve of digestion being followed by amino nitrogen determinations, so that it is possible to produce at will a digest in which hydrolysis has reached 10, 20, 30, or 40 per cent of completion to the amino acid stage. The digestion is stopped at the desired stage by addition of acid, and after autoclaving the solution is filtered and the hydrogen ion concentration brought to the point desired

for pneumococcus cultures. Dr. Avery is about to test media in which peptones of varying degrees of hydrolysis, prepared as above described, are used as nitrogenous foods for the bacteria.

Dr. Stadie and Capt. Robinson have been studying the blood gases in patients with influenza and pneumonia, the oxygen and carbon dioxide in not only the venous, but also the arterial blood being determined. The methods for the determinations are those recently devised by Dr. Van Slyke. Normally the arterial blood seems to be completely or almost completely, saturated with oxygen, although the number of analyses of normal blood is not sufficiently great to make this certain. Influenza patients with cyanosis frequently show as low as 75 per cent of their arterial hemoglobin saturated with oxygen, and in one fatal case, only 25 per cent. The loss of oxygen which the blood suffers in passing from arteries to veins is in some cases also greater than usual, indicating the probability of a slow circulation, which in such cases would also be a factor in producing the cyanosis. The above conditions are indicated rather than proven, for the work is in its preliminary stage; but it appears to promise results of both pathological and physiological interest. The work is a logical outgrowth of that done in the Hospital a year ago by Lundsgaard on the oxygen unsaturation of the venous blood in normal persons and cardiac patients.