REPORT OF THE

DIRECTOR OF THE HOSPITAL

June 1919

TO THE BOARD OF SCIENTIFIC DIRECTORS

OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

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

Gentlemen:

The effect of war on the Hospital was to gradually change its chief activities from investigation to teaching and the care of patients. With the coming of peace it has been possible fortunately to reverse this tendency, and at the present time the Hospital is rapidly getting back to a pre-war basis. The army officers assigned to the Hospital by the Surgeon General's office to act as internes have been ordered elsewhere, and the members of our staff on duty here have all been discharged from the service. Some of the members of the staff who left the Hospital to enter the army have returned, others will not return.

The resident staff has been most seriously affected by the war and the problems connected with the reorganization of this part of the service are among the most important that we have to consider. In the past, as far as possible, only those men were appointed assistant resident physicians who had completed their hospital service and who had had special training in the methods of one of the sciences contributing to medical progress. During the past two years very few young men have been receiving this kind of training. Even the hospital training received by the younger men in the army camps is not that which best fits them for the work to be undertaken here. For these reasons it

is not likely that the work done by the junior members of the staff during the next few years will be so satisfactory as in the past. Moreover, the older members of the staff must devote more attention than formerly to the question of training the young men.

We have been fortunate in obtaining as Resident Physician, Dr. Robert Levy, who has had an excellent training in hospital work in the Johns Hopkins Hospital and who has already carried on a number of scientific investigations, so that he is fitted to direct the work of the assistants in the care of patients and to take a sympathetic interest in their scientific work as well as to carry on his own investigation.

Three assistant resident physicians have been appointed to serve for the remainder of the current year. Dr. William Stadie, who has acted as resident physician during the past year, continues as assistant resident physician. Dr. Arthur Lyon, who was sent here by the army and has been discharged from the service, remains to act as assistant resident physician, and Dr. James Trask has been appointed to act in a similar capacity. It is probable that some of these men will remain during the coming year.

During the period of reorganisation it has been thought advisable to cut down as far as possible the number of patients admitted for treatment. This enables reorganization of the research activities to be undertaken. The study of the treatment of syphilis has been discontinued for the present, and the number of patients suffering from pneumonia admitted for treatment has been reduced. This permits the clinical work to be carried on by the small number of residents I have mentioned and also permits delay in the appointment of other members of the resident staff until men of the highest promise can be obtained.

The following reports indicate the activities which have been under way or are being undertaken by the members of the Hospital staff.

Influenza and Pneumonia.

After the first of January, the acute cases of influenza applying for admission became so atypical that the admission of cases without pulmonary involvement was discontinued and since then only cases showing signs of pneumonia have been admitted. A few of these cases have been typical lobar pneumonia such as those seen in previous years with high leucocytosis, lobar consolidation etc. Most of the cases of pneumonia, however, have been very atypical, run an irregular course, show low leucocyte counts, irregular distribution of the consolidated areas in the lungs etc., therefore having symptoms, signs and lesions very like the cases of influenzal pneumonia seen during the height of the epidemic. Some of these cases have given a history of onset resembling that of acute influenza, in others no such history has been obtained.

One of the chief problems has been to investigate the relationship of influenza bacilli to the epidemic disease and to the pneumonia occurring during the epidemic and later. Unfortunately we have no exact knowledge of conditions before the epidemic as regards the occurrence of B. influensae in cases of pneumonia or their occurrence in normal throats. Observations made during the past winter show that the cultivation of these organisms is made with considerable difficulty and that no comparison can be made between statistical studies made by different observers or even between studies made by the same observers at different times and places. The sodium oleate medium described in my last report has proved of very great value in isolating these organisms, but it has been found that even of this medium

different lots may show marked differences in suitability for growth. Investigations are now under way to determine, if possible, upon what these differences depend.

By making repeated cultures and cultures in various ways, it has been possible to isolate influenza bacilli from the pharynx or sputum or respiratory tract in over 85 per cent of the cases of acute respiratory disease, not only during the severe epidemic, but in the after period as well. In the cases with pneumonia in which cultures were made by lung puncture or at autopsy, influenza bacilli were cultivated from all, never alone however, but always in association with pneumococcus, streptococcus, or staphylococcus.

It will be remembered that during the September - December 1918 quarter, during the height of the epidemic of influenza, a study was made by Dr. Stillman and Miss Pritchett of the presence of B. influenzae in the mouths of the normal persons associated with the Institute, laboratory workers, nurses, doctors etc. At that time 42 per cent of the personnel were found to be harboring influenza bacilli in the throats. This fact seemed of sufficient importance to justify repeated examinations of the throats of these same persons to find whether this percentage of persons remained carriers, whether persistent carriers occurred etc. Consequently during each month a study has been made with the following results:

Each month cultures were made from the throats of 150 or more of the Institute staff and personnel. In January, 11 per cent of the individuals were found to be carrying influenza bacilli, in February, 36 per cent, in March, 50 per cent, in April, 36 per cent. It is possible that the low incidence in January and the high incidence in March may be in part explained by variations in the medium employed. The results show, nevertheless, that a large number of the individuals in the Institute during the present winter have been chronic carriers of this organism. From some

individuals all of the cultures made have been positive; from others the cultures have been persistently negative; while still other individuals, who at first were negative, later became carriers, and vice versa. Investigations were made to show whether the cultivation of the organism from the throat might depend upon some incidental factor; for instance, whether cultures might be positive and cultures made a few hours later might be negative. The evidence so far obtained indicates that this is not the case, but indicates that the technique employed may be relied upon to show whether or not a person is a carrier of influenza bacilli.

Investigations were also made to determine from what part of the naso-pharynx the organisms were most readily isolated, or most frequently encountered. In a series of about 90 individuals three cultures were made from the throat - one culture from each tonsil and one from the posterior wall of the pharynx. In five individuals positive cultures were obtained on plates from all three sources. In five individuals the cultures from the pharynx and from one or the other tonsil were positive. In 13 individuals only the culture from the pharynx showed a growth of influenza bacilli. In no case were the organisms isolated from the tonsil and not from the pharynx. In these 90 individuals, therefore, positive cultures were obtained from 23 or 26 per cent, and the results show that the organisms were most likely to be encountered on the posterior wall of the pharynx. Consequently in most of our cultures since these observations were made, and in most of the cultures before, the cultures were made by touching the posterior wall of the pharynx alone.

In tabulating the results, the persons who gave a history of having had influenza during the present epidemic were separated from those persons from whom no such history could be obtained. The percentage of carriers of influenza bacilli did not differ in these two groups of individuals. The results of cultures from the sick and the well, as mentioned above, indicate that during the epidemic of acute respiratory disease

of the past winter, influenza bacilli could be cultivated from a much larger proportion of the persons suffering from influenza or acute respiratory disease than from the healthy. Doubt might be thrown on this statement if we depended entirely upon the observations of the physicians in the wards, since in these cases a very great effort was made to isolate influenza bacilli in every case, while cultures from the healthy persons were made in a more or less routine manner. However, Miss Winchell, who made the routine cultures from the normal individuals, also made studies in a series of patients, making the cultures in exactly the same way as they were made from the healthy. Whereas among the well persons influenza bacilli were cultivated only from 30 to 40 per cent of individuals, they were obtained by exactly the same technique from over 85 per cent of the cases in the wards. There can be no doubt, therefore, that during the epidemic of influenza of the past winter influenza bacilli have have been frequently present in the mouths of healthy persons living in New York and almost invariably present in patients suffering from acute respiratory disease. Further than this we cannot go at the present time.

Whether during the previous winter, or whether during subsequent winters when no epidemic is present, similar conditions existed or will exist, cannot be stated. However, from our experience, it does not seem probable that influenza bacilli were previously present to an extent at all approaching that seen during the present winter.

Dr. Stillman and Miss Winchell have also made observations concerning the prevalence of influenza bacilli in two institutions near New York. At the New York State Training School for Girls at Hudson, N. Y., influenza prevailed to a considerable extent during January and early in February. On February 16th cultures were made from the throats of 52 patients still in the hospital, but convalescent. From these 52 patients influenza bacilli were cultivated from 20, or 38 per cent. One of the cottages at this institution had been under close quarantine since October 1918 on

account of trachoma. No case of influenza had developed in this cottage. Cultures were made from 20 inmates of this cottage and of these 5, or 25 per cent, showed B. influenzae.

Cultures were also made from a number of children at Mt. Loretta, a Catholic institution for children on Staten Island. This institution was said to have been under strict quarantine during the influenza epidemic, but investigation showed that the quarantine had been quite lax. The employees went to town from time to time and the doctor and priest were frequent visitors to the institution, and the population was more or less shifting. No definite history was obtained, however, of the occurrence of influenza. Cultures were made from 190 of the children, and from 74, or 39 per cent, influenza bacilli were cultivated. The results of all these studies indicate that it will be very difficult to demonstrate any etiologic relation of influenza bacillus to this type of infection, unless it can be shown that influenza bacilli are not all identical, but that different types or varieties exist.

The attempts to learn whether or not this is the case have taken two main directions. First, Dr. Lyon has been investigating the matter by means of immunological methods. The difficulties, however, in the way of carrying out this investigation are very great, probably as great as they have been found to be in similar studies concerning streptococci, but we are still attempting measures which it is hoped may overcome these difficulties.

In the second place, Miss Winchell has been employing the method of sugar fermentation and studying the final hydrogen ion concentration in a number of different strains, hoping that in this way differential characters for different varieties can be found. This work will be reported upon later.

Dr. E. G. Stillman.

The work on the persistence in the human throat of hemolytic streptococci derived from milk and cheese has been concluded. The milk streptococci are not likely to be confused with human streptococci because of the slight degree of hemolysis which the former produce on the surface of blood agar plates. Throat cultures made on the surface of blood agar plates from 40 persons who had just ingested milk known to contain hemolytic streptococci, showed no hemolytic colonies, although in every instance the deep blood agar plates from the same individuals showed the presence of hemolytic streptococci. The cheese streptococci are more likely to lead to confusion as they cause marked hemolysis on the surface of blood agar plates. However, these organisms only persist in the throat for a short time. Although cultures made immediately after eating cheese containing hemolytic streptococci show the presence of these organisms, in cultures made after a few hours, especially if in the meantime other food be eaten, no colonies of hemolytic streptococci are present on the plates. If in any case doubt exists as to the origin of hemolytic streptococci found on blood agar plates, this doubt can be dispelled by testing the acid production of the organisms found, or by studying their growth on agar plates containing methylene blue. Avery has shown that whereas hemolytic streptococci from human sources and those found in milk do not grow on plates containing methylene blue in a dilution of 1:20,000, the cheese streptococci readily grow on such a medium.

The studies concerning the relation of the inhalation of dust to the development of pneumonia in guinea pigs are being continued.

A rearrangement and collection of all the pathological material accumulated since the opening of the hospital is being made. Card catalogues are being prepared which will facilitate the use of this material.

Dr. A. R. Dochez and Dr. O. T. Avery.

During the past three months, in collaboration with Dr. Cullen, a study has been made of the final hydrogen ion concentration of pneumococcus cultures. Previous work by Dr. Avery on the fermentative activities of pneumococci of the different types in media containing various carbohydrates, indicated that no correlation existed between the biochemical activities and antigenic properties of pneumococcus,- it being found that no biologic type of this organism specifically fermented any particular carbohydrate. Since the final and optimum hydrogen ion concentrations for growth of bacterial races are apparently as definite as those for enzyme action, it seemed desirable to fix these values for the different types of pneumococcus. In a previous study by Dernby and Avery it was found that the optimum hydrogen ion concentration for growth of pneumococcus is a pH value of 7.8, and the application of this fact in the adjustment of the initial reaction of culture media has greatly facilitated the cultivation of this organism.

From the present study there has developed the fact that the final hydrogen ion concentration reached by pneumococcus is likewise a biologic constant. When grown in plain broth the pneumococcus reaches a final hydrogen ion concentration of 7.0, while in dextrose broth the limiting value is about pH = 5. Experiments show that these final hydrogen ion concentrations are the same for all types of pneumococcus and are constant without regard to the initial reaction of the medium or the concentration of sugar used.

Thirty-five strains of pneumococcus, representing different types and isolated from various sources, some of which had been cultivated on artificial media for years, and others of which were removed but one or two transfers from active disease processes, all reached the same final hydrogen ion concentration in 1 per cent dextrose broth, i.e. pH = 5. This is precisely the point which limits the growth of pathogenic hemolytic

streptococci of human origin, when grown under similar conditions.

It has been found further that when pneumococci of different types were grown in media containing other fermentable carbohydrates such as lactose, saccharose, maltose, galactose, raffinose and inulin, that the same final hydrogen ion concentration was reached by all types. As far as tested no type of pneumococcus has been found to split mannit, arabinose, or rhamnose.

Work is being continued on the use in culture media of soaps of the unsaturated fatty acids, such as sodium oleate. It seemed desirable to determine the relation of the degree of unsaturation to the growth accelerating properties of soaps for certain Gram negative organisms, especially B. influenzae, as well as the relation of unsaturation to the bactericidal action of soaps for pneumococci and streptococci. To this end Dr. Cullen has undertaken the chemical analysis of the various samples of sodium oleate which are being used in these bacteriological experiments. It has been found that pure oleic acid in concentration of 1:100 in culture medium, the reaction of which has been readjusted, permits the influenza bacillus to grow luxuriously, while growth of pneumococcus and streptococcus is restrained at a dilution of 1:5000 in solid medium and in fluid medium. The initial growth is inhibited in dilutions of oleate as high as 1:100,000. It is of interest that a culture of B. influenzae grown in oleate broth 1:500 was found viable after three months' incubation at 37°.

In working with medium to which higher concentrations of oleate are added it has been found essential to determine the reaction of the medium, since ionization of the soap tends to unduly increase alkalinity and inhibit growth.

Study of Hemolytic Streptococci.

Since our last report, study of the antigenic relationship of strains of hemolytic streptococcus has been continued. The result of this work has been to confirm and extend the information already acquired, so that now it appears that certain different types of this organism exist and that these types can be sharply differentiated one from the other by meanns of agglutination and protection reactions. In all 141 strains have been studied and of these, 96 or approximately 70 per cent, fall into definite types and 45 are as yet unclassified. The 96 classifiable strains comprise six types; type "S 3" consisting of 28 strains, type "S 23" of 20 strains, type "S 60" of 28 strains, type "S 84" of 9 strains, type "S 32" of 5 strains, and type "S 273" of 6 strains. Study of the cross immune reactions of the first four types has been completed and that of the last two types is in process of investigation.

Agglutination.

The immune sera used in the agglutination and protection tests, were obtained by the immunization of rabbits, sheep and dogs. The animals were inoculated intravenously with repeated doses of heat-killed organisms, and in most instances a certain number of doses of living organisms were given. It is questionable whether the use of living organisms is essential to the production of a potent serum. The loss of animals with such a method either from general infection or local infection of joints is considerable. The employment for immunization of freshly isolated, unpassed human strains, or the use of the same strains after a series of animal passages, does not alter in any recognisable way the specific qualities of the serum.

The agglutinin and protective titre of the sera has remained undiminished for many months after the time of bleeding. Great care is taken in the preparation of the organisms to be used in the agglutination reaction. The broth is made from carefully selected meat, and

instead of the usual sodium chloride a sufficient quantity of a balanced phosphate mixture is added to give the desired salt concentration and to adjust the hydrogen ion concentration to a pH 7.4. Experience has taught us that when no sugar is added to the medium, S. hemolyticus grown for 24 hours in such a culture fluid does not develop an acidity greater than pH 7.2, which is just above the point at which granulation appears. The organisms are removed from the culture medium by centrifugalization and are washed once or twice in a broth of pH 7.4 prepared in the manner described above. They are then resuspended in the same medium in a concentration approximately that attained after 24 hours growth, and are ready for use in the reaction. The sera to be employed in the test are made up to the various concentrations by dilution with the same broth used for suspending the bacteria. The reaction of each specific serum is controlled by a complete series of like diultions of normal serum of the same animal. Equal quantities of serum dilution and bacterial suspension are added to each tube and the reactions are then placed at a temperature of 55° C. for one hour. A temperature of 55° must be accurately maintained during the period of incubation and the test must be read after the lapse of one hour, since if they are allowed to continue longer, non-specific granulation occurs.

If clumping develops in the broth controls, or in more than the first two or three dilutions of normal serum, the reaction is to be regarded as unsatisfactory and discarded. By the use of this technique it has been possible to carry out reliable agglutination tests of S. hemolyticus and to show that constant type relationships exist and that the various types are sharply differentiated from one another. There is little or no cross-agglutination of the various types in heterologous sera, even in a concentration of 1:20. Homologous sera agglutinate organisms of the same type in dilutions ranging from 1:1200 to 1:5000, and practically all strains of the same type react to the same point.

Protection.

Although the classification of S. hemolyticus by means of the agglutination reaction has been presented first, we have actually obtained our primary indications of antigenic differences between strains by means of the reaction of protection. Later each reaction has been used to confirm the information obtained by means of the other. In the successful carrying out of protection experiments, two things are of especial importance; first, the production of a serum of high potency, and second, the possibility of raising the virulence of the test strains of streptococcus to such a point that very minute doses of culture are sufficient to kill white mice in a limited period of time. The virulence of hemolytic streptococci for the ordinary laboratory animals is low when compared with an organism like pneumococcus. Doses of one cubic centimeter or more of a twenty-four hour broth culture administered intraperitoneally are required to kill guinea pigs and rabbits. Furthermore, repeated passages through these animals fail to bring about a considerable accession of virulence. The fatal dose for white rats and mice is smaller, usually in the neighborhood of 0.1 cc. of a broth culture. It has been possible to raise the virulence of many strains by continuous passage through white mice and rats to such a point that doses of from 0.000001 cc. to 0.00000001 cc. of broth culture are sufficient to kill the former animals in from twenty-four to forty-eight hours.

We have been able to produce sera in the manner alluded to above of such potency that 0.5 cc. administered intraperitoneally is sufficient to protect a white mouse against one hundred thousand lethal doses of a highly virulent streptococcus. In order to produce such a serum many animals must be used, only a few of which may give the desired result. These seemingly difficult conditions must be attained in order that sufficiently long-range protection titers may be carried out to insure the reliability of the information obtained. Protection against one or two lethal

doses of a series of strains of streptococcus by a monovalent serum is subject to such a variety of interpretations that the evidence so gained cannot be considered of much value in judging accurately of antigenic relationships of the different strains. The technique observed in the protocols has been as follows: The potency of all sera has been tested for the homologous strain of organism and only such sera utilized as gave a sufficiently wide range of protection to insure dependable results. For infection virulent streptococci have been used which have been grown for approximately eighteen hours in either plain broth or ascites broth. In the inoculation of animals, the technique advised by Neufeld has been followed with only a minor variation. The test animals have been injected intraperitoneally with 0.5 cc. of serum twenty-four hours before the conduction of the experiment. Tentative trials have shown that if the serum is given simultaneously with the infecting dose, no protection results, and that to ensure success the serum must be given at least eight hours before infection. On the following day a series of virulence controls are inoculated intraperitoneally and the serum animals injected in the same manner with doses of culture ranging from 0.001 cc. to 0.00000001 cc. of broth culture. Animals surviving for a period of five days are considered to be adequately protected. By the use of this method it has been possible to test the antigenic relationship of a considerable number of virulent strains of S. hemolyticus. In every instance in which it has been possible to carry out protection tests the same type specificity has been manifest that was evident from the agglutination experiments.

This work has cleared up a number of points about Streptococcus hemolyticus that have been in dispute for many years. In the first place, S. hemolyticus is not a unit type as was previously supposed, but probably consists of a number of types, at least six of which have been identified. It has been possible to produce sera of considerably higher potency than any hitherto reported. Previous investigators have stated that

freshly isolated human strains change their antigenic properties on animal passages, and that the latter procedure developes what has been called "animal virulence" and gives a common antigenic character to all strains. We have found no evidence to support this contention, in fact immune sera produced with human strains that have never been passed through animals afford a high degree of protection against strains that have received many animal passages. In addition, the antigenic difference between strains of S. hemolyticus which have been passed through animals is quite as distinct as that between strains which have not been so passed.

It appears from this work that there is some relationship between fermentative and antigenic characters. All the members of type "S 60" ferment mannit, and there are no mannit fermenters in any of the other groups. There are, however, two strains which ferment mannit which are unclassified.

By this work a method has been developed for the identification and classification of S. hemolyticus, and a satisfactory method has been found for the titration of antistreptococcus serum. Thus the way has been cleared for epidemiological investigation and for study of specific therapy and prophylaxis.

Dr. M. A. Barber.

It will be recalled that in a previous report attention was called to work by Dr. Dochez indicating that antipneumococcus immune serum caused some inhibition of growth of the homologous organisms. This effect was correlated with the so-called antiblastic immunity. Later observations by Dr. Blake throw some doubt on these conclusions and the matter has remained unsettled. It seemed possible that the question might be definitely answered by using the technique developed by Dr. Barber for the isolation and growth of single cells.

Dr. Barber has made an extended series of observations

observing the division time and rate of growth of single pairs of pneumococci when placed in immune serum and under diverse other conditions.

The conclusions from these observations are:

1. In homologous serum pneumococcus becomes invested with a thick capsule and tends to grow in chains or zoogloea-like masses.

2. In heterologous serum the growth is similar to that in broth.

3. Growth rate in homologous serum during earlier generations is apparently exactly the same as that in the heterologous serum or normal horse serum.

In connection with the experiments on pneumococcus the growth rate of this organism was investigated. In hanging drops when conditions are most favorable, the generation time is approximately 30 minutes. Where one pair or similar small sowings are made into test tubes containing 5 to 10 cc. of medium, the rate is practically the same, about 30 generations in 15 hours. From the time when the first appreciable cloudiness appears in a test tube until the tube is distinctly cloudy, the rate is also 30 minutes. During the first half hour after isolation in a hanging drop one generation is formed and two during the first hour. Some experiments indicated that a somewhat more rapid growth (generation time 26 to 28 minutes) takes place in capillary tubes. This observation will be confirmed.

Another phase in the growth of pneumococcus is being investigated, the lag, or in some cases failure to grow, of single pairs, or similar small sowings, made into test tubes containing relatively large quantities of broth, when controls in hanging drops or on agar show growth. Twenty-eight to thirty series, including some 325 test tube cultures have been done.

General summary of results:

Of some 325 transfers approximately 50 per cent have grown. In some later experiments with plain broth nearly 90 per cent have grown. A pair, or even a half-pair, transferred when actively growing, may

continue growth in the test tube at an undiminished rate, at all events, may accomplish about 30 generations in 15 hours.

Presence of serum increases probability of growth.

.rently does not.

Some series indicate that contact with the sides or bottom of the test tube or with some solid substance promotes growth. This has not been definitely proved.

The period of growth at which cells for sowing are taken is undoubtedly an important factor. Washing in broth of cells from an old culture apparently promotes their chances of growth in test tube or hanging drop.

It is doubtful whether the quantity of fluid to which cells are transferred is in itself an important factor. Growth has succeeded further in 125 cc., where one pair was sown. This matter will be tested with large quantities of medium.

Dr. D. D. Van Slyke

Improvement in the McLean-Van Slyke Blood Chloride Method.

The micro-method of McLean and Van Slyke for the determination of blood chlorides required three filtrations - one after coagulation of the proteins with heat and magnesium sulfate, a second after treatment of the filtrate with charcoal, and a third after precipitation of the chloride with standard silver nitrate. In the final filtrate the excess silver was determined by our iodometric titration.

A modification of the method was made necessary by the lack of chloride-free magnesium sulfate and charcoal during the war.

With the assistance of Lt. James Donleavy, one of the instructors in the U. S. A. class in clinical chemistry, the necessity for these reagents was obviated, and at the same time the three filtrations were condensed into one. By adding picric acid to the standard silver nitrate a solution was obtained which precipitates both the proteins and the chlorides of the plasma simultaneously. The determination is therefore simplified to a single filtration, and titration of the filtrate.

Method for Titration of Organic Acids in Urine.

While Dr. Palmer was on the staff a method for titration of organic acids in the urine was partially perfected. Dr. Palmer left before the method could be finished, and it has recently been taken up and brought to an apparently practical condition. Phosphates and carbonates are removed by shaking the urine with calcium hydroxide and filtering. To 25 cc. of the filtrate N/5 HCl is added until the alkaline solution becomes neutral to phenolphthalein, the reaction being pH = 8. The actual titration is now begun. Tropeolin OO is added as indicator and N/5 HCl is added from a burette until a pH of 2.7 is reached. At pH = 8 all organic acids occurring in urine are in the form of salts, at pH = 2.7 they are from 93 to 100 per cent free. The amount of HCl added to change the reaction from pH = 8 to pH = 2.7

therefore indicates approximately 96 per cent of the organic acids present.

If weak nitrogenous bases were present they would also be estimated in the titration. The only such base occurring in urine in significant amounts is creatinine, which is included quantitatively in the titration. Consequently the organic acid titration is, for exact results, to be corrected by subtracting the amount of creatin present in terms of M/10 solution.

The total output of organic acids in a normal adult appears to be 500 to 800 cc. of N/10 acid. In diabetic ketonuria the output rises parallel with the acetone bodies, and may reach several times the normal value. The titration is extremely simple and may be used as an approximate measure of the ketone excretion in diabetics when facilities are not present for the exact determination of the ketone substances. It appears possible that information on several other problems may be gained with the method.

Titration Method for Plasma Bicarbonate.

With Dr. Stillman and Dr. Cullen a method has been devised for determining the plasma bicarbonate by simple titration. The results are quantitatively identical with and equal in accuracy to those obtained by determining the CO_2 combining power of the plasma. The new method, although rendered possible only by the physico-chemical studies on blood reaction published in the past few years, is extremely simple and requires no special apparatus. The blood plasma (2 cc.) is mixed in a relatively large flask with an excess of N/50 HCl (5 cc.), and the carbon dioxide set free by the acid is permitted to escape by whirling the solution around the inner wall of the flask for about one minute. Neutral red is then added as indicator, and N/50 sodium hydroxide is run in from a burette until the color equals that of a standard phosphate solution of pH 7.4, the reaction of the blood in vivo. The use of this endpoint assures that the proteins bind only the same amounts of acid and alkali as in vivo, and do not affect the accuracy of the titration. The

difference between the 5 cc. of N/50 HCl added to the plasma and the N/50 NaOH required to titrate back to normal blood reaction represents the amount of acid neutralized by the plasma bicarbonate. The titration has an advantage over the gasometric CO_2 method in that the titration requires no special apparatus, although it is no quicker, and does require accurate standard solutions of acid, alkali and phosphate.

Study of the Hemoglobin Changes in Pneumonia.

Dr. Stadie has added further observations to his previous results, all confirming the latter in showing that cyanosis in pneumonia is due to incomplete oxygenation of the arterial blood, and that a low oxygen saturation of the arterial blood is of prognostic importance.

Dr. Stadie, for the study of the methemoglobin question in pneumonia, has also devised a method for determining methemoglobin, which, while not yet adequately tested, appears promising. The unchanged hemoglobin is estimated from the combining power of the blood for oxygen, determined gasometrically. The unchanged hemoglobin plus methemoglobin is estimated by treating the blood first with potassium ferricyanide, which turns all the hemoglobin to methemoglobin, then with potassium cyanide, which turns all into cyanhemoglobin. The latter is determined colorimetrically. The difference between this value and the hemoglobin estimated from the oxygen capacity represents the methemoglobin.

The Distribution of Arsenic in the Body after Intravenous Injection of A 189, and the Mode and Rate of Excretion.

This work, which was in progress at the time of the last report, has been completed with Dr. Stadie. The following summarizes the results:

1. The excretion of A 189 in the urine of man, as measured by the total arsenic, is practically complete at the end of twenty to thirty days.

2. The total excretion of arsenic in the feces is equal to or greater

than the excretion in the urine.

3. Arsenic is excreted in considerable quantities in the stomach.

4. In the excretion of arsenic after intravenous injection of A 189, rabbits resemble man in that they eliminate about equally through the kidneys and intestines and require about three weeks to free their bodies of the injected arsenic, when the latter has been given in large amounts. When the arsenic excretion is complete, the arsenic content of the organs of the rabbit is no higher than in controls which have recieved no A 189. There is no permanent deposition of arsenic in the tissues.

5. In the blood, A 189 is almost entirely in the plasma and serum. Concentration in the blood falls off very rapidly after intravenous injection. At the end of an hour, the average concentration is only 2 to 10 mgms. of arsenic per liter.

6. No arsenic was found in the spinal fluid of five treated cases.

7. In the tissues of rabbits injected with non-fatal doses of A 189, the highest concentration of arsenic is in the liver, the kidneys and muscles coming next. The brain contained very little.

8. After doses of 50 mg. per kilo (sub-lethal) no difference was observed in the distribution of arsenic in the tissues, whether the A 189 was injected with one or three molecules of sodium hydroxide.

9. Injection into a rabbit during two to five minutes of 150 mgms. of A 189 per kilo with one molecule of sodium hydroxide kills the rabbit with an extremely high concentration of arsenic in the lungs, apparently due to flocculation of A 189 in the pulmonary capillaries. In two experiments the injection period was prolonged to 30 minutes, and the rabbits were uninjured. This suggests that reduction of the alkali from 3 molecules to 1 molecule does not increase the toxicity of the drug, except insofar as it favors immediate flocculation in the capillaries.

10. Solutions of A 189, when mixed with blood serum or plasma, give

rise to a precipitate over a wide range of dilutions. The solubility of A 189 in serum is such that a liter of saturated serum contains 0.35 mgm. of arsenic. The solubility is much less than that of salvarsan. When the drug is mixed with a large volume of serum the solubility is not affected by the amount of alkali (1 or 3 molecules) with which it is dissolved before mixing with the serum.

11. Since, after injection of A 189 in therapeutic doses, from 6 to 15 mg. of arsenic per liter are found in the blood, the serum of which can dissolve only 0.35 mg. per liter, it seems that the injected drug, unless immediately altered to a more soluble product, must circulate in the form of a suspension, a relatively small part being in true solution. That the arsenical fraction at least ultimately does alter into a more soluble product is indicated by the fact that the arsenic concentration in the urine may reach 10 mg. per liter.

Study of the Therapeutic Effect of A 189 on Human Syphilis.

This work was begun in January 1918 by Dr. Stillman. Dr. Stadie joined the problem about three months later. During the past fall Capt. Klauder and Lt. Fortuine of the Sanitary Corps were detailed to the Institute to assist in the care of the patients. The problem in this Hospital was closed, for the present at least, after one year's work, in January of the present year. The results are summarized as follows:

I. Experiments in mode of administration. After preliminary experiments, the drug was systematically administered according to the following plans, which are given chronologically.

1. Without mercury.

a. 10 mg. of A 189 per kilogram every 5 days. Therapeutically efficient but abandoned for less intensive treatment because of abdominal pains.

b. 7.5 mg. of A 189 per kilogram every 7 days.

Therapeutically efficient, almost no cramps; but 3 cases of exfoliative dermatitis. Therefore abandoned for the following:

2. With mercury.

a. 7 mg. of A 189 per kilogram every 14 days, with mercury either as $HgCl_2$ intramuscularly, or as metallic mercury inunctions. No serious toxic symptoms of any kind in 11 patients treated. Wassermann reaction disappeared after the administration of but half as much A 189 (20-40 mg. per kilo.) as was required when A 189 was given alone. Two cases during treatment developed mucous patches which cleared up on continuation of the same treatment.

II. Therapeutic results. Eighty cases of syphilis have been treated with A 189 until they were either free of lesions and Wassermann reaction, or showed toxic symptoms sufficient to make cessation of treatment advisable. Of the 80 cases, 21 were primary, 37 secondary, 3 tertiary, 8 latent, and 11 central nervous syphilis.

Time did not permit a serious attempt to cure the central nervous cases, although 3 of them were cured, both in symptoms and in the Wassermann reaction of the spinal fluid.

Of the 69 other cases the treatment was successful in 66, both Wassermann reaction and lesions disappearing. One primary case free from Wassermann reaction at the beginning remained free.

The 3 remaining cases failed to tolerate sufficient A 189 to make them Wassermann-free. The treatment was stopped in one case because of abdominal cramps and bloody stools, and in another because ofexacerbation of an already existing retrobulbar neuritis. The third failure to complete the treatment was due to exfoliative dermatitis which resulted fatally.

It is a point of interest that the 3 cases of tertiary syphilis were cured, by A 189 without mercury, of lesions and Wassermann reaction as rapidly and with as little drug (50 mg. per kilo.) as were the primary cases.

With so few cases one cannot, of course, say whether such results may be expected again or are exceptional responses.

The results above summarized were obtained while the optimum mode of administration was being worked out. Consequently most of the cases reported were not treated according to what present experience indicates as the plan involving minimum toxic symptoms with efficient therapy.

III. Suggested mode of administration. The results summarized above under "Experiments in Mode of Administration" indicate that the optimum treatment with A 189 probably lies between Plan 1 b, which appeared too intensive (occasional toxic symptoms) and Plan 2 a, which appeared not intensive enough (occasional recurrence of lesions during treatment). It was, therefore, planned to test the following:

7 mg. of A 189 per kilogram body weight every 10 days, the A 189 being prepared for intravenous injection by solution in 1.00 molecule of NaOH and 300 parts of 0.9 per cent sodium chloride solution. Simultaneous treatment with mercury.

Improvement in the Van Slyke Method for the Hydrolysis of Proteins. Miss Hiller.

This method was published in 1911, and has in the past few years been much used in protein analyses, particularly of protein foods. The amino acids are divided into two groups, the hexone bases and the mono-amino acids, by precipitation with phospho-tungstic acid. The bases, arginine, histidine, and lysine, are determined by methods which have stood the test of use satisfactorily. The mono-amino fraction is divided into two sub-groups by determination of total nitrogen and free amino nitrogen. The sub-group containing non-amino nitrogen consists of the three amino acids, proline, oxyproline, and tryptaphane. This non-amino nitrogen has been determined by difference between the total and amino nitrogen of the mono-amino fraction, and like all determinations by difference was subject to a summation of errors.

Miss Hiller has been working out a direct method to displace the determination of this group by difference. The amino nitrogen is destroyed by nitrous acid and the nitrous acid is gotten rid of by reducing to ammonia with a zinc-copper couple. After boiling off the ammonia the only nitrogen left is that of the non-amino type, and this is determined by Kjeldahl. The method has been developed sufficiently to give accurate results with pure amino acids, and has been applied to the analysis of gelatin and casein with good results.

Miss Hiller is now working on a new method for the determination of histidine in hydrolyzed proteins. The hexone bases, arginine, lysine, and histidine are separated from the other amino acids by precipitation with phosphotungstic acid, and all the nitrogen, except the two non-amino nitrogen atoms of histidine, is eliminated by treating first with alkali, then with nitrous acid, the residual nitrogen representing the histine. The method is not yet complete.

Preparation and Testing of Desoxycholic Acid as the Pneumococcus-Dissolving Substance of Bile.

Miss Hiller has purified by recrystallization a considerable amount of desoxycholic acid, which has been stated by Mair to be the substance responsible for the ability of the bile to dissolve pneumococci. With Dr. Avery the substance is about to be tested in regard to its solvent power for pneumococci.

Dr. Salvesen, a volunteer assistant from Norway, has extended the work on blood gases to carbon monoxide. We have found that the gasometric method for blood oxygen previously worked out in this laboratory can be successfully applied to carbon monoxide without any change except the addition of a short step at the end of the determination. Carbon monoxide and oxygen are extracted and measured together. Then the oxygen is absorbed by letting 1 to 2 cc. of pyrogallol solution flow slowly into the apparatus.

The residual gas, after correction for the slight amount of nitrogen gas dissolved by blood, is measured as carbon monoxide. The method has given entirely reliable results for blood with all possible proportions of carbon monoxide and oxyhemoglobins. Guinea pigs were found to become unconscious when approximately 3/4 of their hemoglobin was changed to carbon monoxide hemoglobin. It appears to be the first carbon monoxide method sufficiently simple and accurate to make clinical application possible.

Dr. Salvesen is about to apply this method in an attempt to improve Haldane's technique for determining the blood volume in animals and man. The subject is allowed to absorb into his blood a known amount of carbon monoxide gas, and the amount present per cc. of blood is determined. The result indicates the total blood volume.

Dr. Salvesen also hopes to check up more accurately the experiments of Haldane on carbon monoxide absorption, which form the sole basis for the oxygen secretion theory, which postulates that oxygen may be actively secreted by the lungs into the blood, so that the oxygen tension in the arterial blood may exceed that of the alveolar air. Krogh has severely damaged this theory with his tonometer experiments, but no one has yet demonstrated any fallacy in Haldane's experimental results, or repeated them with improved technique such as would justify either their rejection or their acceptance as experimental facts.

Dr. A. Cohn

I returned to the Hospital of The Rockefeller Institute on February 1, 1919, after an absence of exactly one year. From February 1 to March 12, 1918, I was on duty in the Office of the Surgeon General. In this post, the preliminary organization of U. S. General Hospital #9 engaged a large portion of my attention. On March 15 I sailed to join the American Expeditionary Forces. After conferences in England, I became an assistant of Major (later Brigadier General) Thayer and bore the title of

Senior Consultant in Cardio-Vascular Diseases, A. E. F.

Experience with a great many invalided soldiers showed that the cardiac complaints, which it was my duty to study and to suggest management for, depended not on a structural disease of the heart, but represented an expression, the usual expression, of a psycho-neurotic or an anxiety state. To limit all cases of cardiac complaints to an affection of this nature is however incorrect. Improperly managed convalescents from acute infectious diseases who complain in a similar fashion are suffering from a different condition and require different management. This is also true of men who have been obliged to undergo exertion beyond their ability to perform work. They belong probably to the group of cases of "Heart Strain". These groups were confused in the minds of medical officers and required study to separate them rationally with a view to proper therapy.

On the nature of these various affections, information was gathered also by Dr. Francis W. Peabody at the Lakewood Hospital. The experiences and observations of both of us are now being collected, and will be published as a book. We hope the book may show what advances have been made during the war of 1914-18 in understanding this rather complicated subject. We think it should also present whatever serious contributions have been made by other observers.

I. A paper on the "Effect on the Rabbit's Heart of Injecting the Drug A 189 and of Injecting Salt Solution" has been completed. Forty-one experiments were done. It was found that 30 mgm. per kgm. of rabbit's weight were without effect on the hearts of four rabbits. When 50 mgm. were injected, 9 of 14 rabbits showed an alteration on the mechanism of the heart beat; one of these rabbits died. When 100 mgm. were injected, 9 of 10 rabbits showed alterations; 9 of these rabbits died. One of those which died was the one showing no effect on the heart.

Injecting salt solution (0.85 per cent) caused

irregularities in 2 of 7 rabbits. These rabbits all recovered.

When N/2 NaOH was added to the salt solution in the same amount as is added when A 189 is present, 4 of 6 rabbits showed changes in the heart. One of these rabbits died.

The conclusions drawn from these experiments are as follows:

1. A 189 is toxic for the hearts of rabbits, when injected at the rate of 50 mgm. per kilo. of rabbit's weight.

2. Rabbits are killed when 100 mgm. per kilo. of body weight are injected.

3. The administration of the drug is followed by a variety of cardiac irregularities.

4. The injection of salt solution and of salt solution to which N/2 NaOH has been added may be followed by cardiac irregularities.

II. We have made daily electrocardiograms of a number of animals, into which Dr. Noguchi has injected the spirochaete of yellow fever. It has been observed that in patients the heart rate in this disease is low. Electrocardiograms have been made primarily to obtain curves of the rate in the experimental animals, and to ascertain the mechanism of the bradycardia, if present.

III. With the cooperation of Major General Shanks it has been possible to study 200 soldiers, chiefly of the Infantry, who have served overseas. So far 143 soldiers have been examined. A physical examination is made before and after a standard exercise test; an electrocardiogram is taken in the recumbent and in the erect positions, together with the curve of respiration; and an X-ray plate is made at two meters, in inspiration, the breathing being normal. A certain number of plates will be made in expiration as well. It is expected that information will be obtained on the following main points:

a. The size of the heart.

b. The position of the heart in the chest.

c. The type of electrocardiogram associated with differences in size and shape of the heart.

d. The influence of respiration on the electrocardiogram, account being taken of the position of the heart in the chest.

e. The effect on the electrocardiogram of position, i.e. of lying and standing.

IV. Dr. Levy has studied with Dr. Cullen the methods of preserving G-Strophanthin in solution. It has been shown that this drug deteriorates in potency if sterilized in glass containers yielding alkali. This deterioration is accompanied by change in optical rotation. It has been found that this deterioration may be prevented by dissolving the drug in a buffer mixture (phosphates) at the neutral point (pH 7.0).

Dr. Levy is also engaged in studying the G-Strophanthin prepared by Jacobs and Heidelberger. So far it has been shown that the action of this drug is identical with that of G-Strophanthin (Merck). This work has been preliminary to further studies on the pharmacology of strophanthin.