

10-20-22

REPORT OF THE DIRECTOR OF THE HOSPITAL

October 20, 1922

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October, 1922.

Gentlemen:

In the report of the work of the Hospital for the preceding year mention was made of the changes in plan of organization dependent upon the greater age of the department and increased experience on the part of certain members of the staff. The separation of the Hospital activities into several divisions has taken place without any serious loss in unity and with an undoubted gain in effectiveness. It is obvious that in clinical work the mutual assistance and cooperation of the various members of the staff is of very great importance; indeed, it is quite necessary in order that the greatest use be made of the available material. The spirit of coordinated effort and interest which is demanded and which has been so evident in the Hospital in the past has not diminished and each member of the staff has been willing to bring his special knowledge to the solution of any problem under investigation in any part of the Hospital. As a result of this, a review, such as the present, describing the activities of the individual groups of workers, may not give full credit to each individual or group. For instance, in the study of nephritis, or in the study of respiratory disease, studies must be made not only of renal functions or of the respiration but frequent and care-

ful studies must be made of the state of the heart as well. Studies of this kind carried on by Dr. Cohn and his associates, not bearing directly on their own problems, receives no special notice in Dr. Cohn's report, though they may have demanded much time and effort and have been of much value in aiding in the solution of the problems under investigation in the Hospital. This fact must be kept in mind when reviewing the achievements of the various groups of workers.

Compared with the previous year many changes have occurred in the staff, but these have occurred without any serious effect on the work of the Hospital. Mention was made in the last report of the appointment of a number of the men to positions in other institutions. The appointment of Dr. Austin, Resident Physician, as Professor of Research Medicine at the University of Pennsylvania did not become effective until January first, so that he was able to continue his duties here until that time. Upon his departure his duties as Resident Physician were taken over by Dr. Lundsgaard. His long experience as resident physician at the Hospital of the University of Copenhagen enabled him to carry on this work here with great effectiveness. However, he desired to be relieved of his administrative duties and wished to reside outside the Hospital, so Dr. Hugh Morgan has been appointed to the post of Resident Physician. Dr. Lundsgaard remains a member of the staff and is engaged especially in the study of nephritis.

Dr. Edgar Stillman, who for many years has been a member of the Hospital staff and has assisted in the work carried on in relation to diabetes, the study of chemo-therapy of syphilis and more recently the study of nephritis, has received an appointment as Associate in Medicine in Columbia University and Assistant Visiting Physician at the Presbyterian Hospital. Dr. Levy, who has been associated with Dr. Cohn in

the study of heart disease, also has left to accept similar positions at Columbia and the Presbyterian Hospital. These changes have necessitated several new appointments. It is a great satisfaction to note that the number of well-trained men who desire to undertake the kind of work for which the Hospital makes provision is much greater now than it was immediately following the war. As a result, it has been possible to complete the staff for next year with a group of well-trained, enthusiastic and, apparently, capable young men.

Two new undertakings are now under way in the Hospital, the results of which, however, will have to wait for a later report. The two developments may be mentioned, however. First, the character of the studies which are being conducted in relation to heart disease is being modified, and this has necessitated a reorganization of the group of men working under Dr. Cohn and a considerable change in the laboratory equipment. Dr. Henry A. Murray, who has received an appointment on the Hospital staff, will undertake the culture of heart tissue in vitro with the purpose of using this growing tissue in the solution of certain problems relating to the physiology and pathology of the heart.

The second important enterprise consists in undertaking the study of chicken-pox. This study is not being conducted because of any very great social or economic importance of the disease itself, but it is believed that further knowledge concerning its etiology and nature may have wide application to our knowledge of other contagious diseases. This work will be carried on directly by Dr. Thomas Rivers, who comes from the Pathological Department of Johns Hopkins University, with the assistance of Dr. William S. Tillett.

With these new projects well under way and with a prom-

ising outlook for progress in the solution of problems previously under investigation great hopes are entertained for the success of the coming year.

Inquiry by visitors and others is frequently made concerning the methods we employ for obtaining patients suitable for the studies being carried on. Very soon after the Hospital was opened it became apparent that there were practical difficulties in the way of obtaining patients through other hospitals and it was found that it would be necessary to depend upon practicing physicians to refer to the Hospital the kind of patients desired. For the purpose of getting into contact with these physicians and of informing them of the types of patients we wish to admit, we began the practice of sending bulletins from time to time to all the physicians in New York. I am attaching the latest Bulletin which gives information concerning the diseases which it is planned to investigate during the coming winter. The response on the part of physicians in the past has been most satisfactory, and on the part of the public there has never been the least evidence of fear or distrust ~~or~~ disinclination to come to this Hospital because one of its functions is to aid in the investigation of disease.

*The Hospital of The Rockefeller Institute for Medical Research confines its work to selected cases that bear upon a limited number of subjects chosen for investigation. It is the practice to issue Bulletins from time to time, giving notice of subjects thus chosen, in order that suitable patients may be referred to the Hospital by physicians and others who are interested and who are willing to co-operate in this way. No charges are made for treatment, room, board, or any other services.*

**HOSPITAL OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH**

66th Street and Avenue A, New York, N. Y.

**BULLETIN OF DISEASES UNDER INVESTIGATION**

September 30, 1922

The following conditions will be the subject of study in this Hospital during the coming winter; patients suffering from these diseases will be admitted to the extent of the Hospital facilities:

**I. Acute Rheumatic Fever.**

- (1) Patients with acute rheumatic polyarthritis.
- (2) Children during the acute stages of recurring endocarditis, pericarditis, or myocarditis of supposed rheumatic origin.
- (3) Patients with chorea and arthritic or cardiac involvement.
- (4) Patients with rheumatic fever showing subcutaneous nodules.

**II. Nephritis.**

Patients suffering from acute nephritis or from chronic nephritis of moderate severity, also a few cases with arterial hypertension unassociated with evidence of kidney disease, will be admitted. Cases of nephritis in young persons, particularly cases showing edema, are especially suitable.

**III. Chicken-Pox.**

Patients with chicken-pox will be admitted to the Isolation Ward of this Hospital for care and treatment. It is desired that patients be referred as early in the disease as possible.

**IV. Acute Pulmonary Infections, including Acute Lobar Pneumonia.**

It is desired to receive patients suffering from atypical forms of acute infection of the respiratory tract as well as those with acute lobar pneumonia. Patients with the latter condition should be referred as early in the disease as possible in order that suitable cases may receive the greatest benefit from serum treatment. A chamber is now available in which patients may be treated in atmospheres rich in oxygen.

**V. Cardiac Disease.**

In continuing the study of the treatment of chronic heart disease, it is planned to admit patients, either young or old, in the more advanced stages of heart failure. Such patients as show completely irregular pulse and are suffering from fibrillation of the auricles are especially desired.

Physicians may communicate with the Hospital by telephone (Rhinelander 0900) or by personal application to the Resident Physician. An ambulance will be sent promptly when required.

Infectious Diseases.

Acute Respiratory Diseases, Including Lobar Pneumonia.

This study began several years ago with an investigation of lobar pneumonia, one of the objects being the development of a specific therapy in this disease.

This investigation resulted in the accumulation of more accurate knowledge concerning the bacteria concerned, the development of methods for determining the bacterial incitant in each individual case and the development of a method of serum treatment effective in the cases associated with the presence of one kind of bacteria. During the progress of these studies it became increasingly evident that lobar pneumonia, of pneumococcus origin, should not be studied as an isolated phenomenon but that it is related, certainly as regards pathogenesis, to a whole series of infections of the respiratory tract, some severe, others very mild in their manifestations. Moreover, during the years this study has been in progress the character of the prevailing pneumonia has varied greatly. This has been due to some extent to the unusual conditions brought about by the war, and also by the prevalence of so-called influenza in epidemic form, but, in addition, other factors have probably also been operating. It was therefore thought necessary to greatly broaden the scope of this inquiry if a clear conception of the nature of this group of diseases was to be obtained. This explains why during the past year the studies which have been pursued have taken several different directions.

Although in the following description the studies may appear somewhat unrelated, nevertheless they represent work necessary to be done before progress may be made in the general problem under investigation.

Studies in Pathogenesis of Pneumonia.

It has come to be generally held that in acute lobar pneumonia infection takes place by way of the respiratory tract. There is some evidence that in the case of pneumonia due to Pneumococcus Type I and Type II. infection is fairly direct, that is, is spread from the sick to the well through fairly direct contact. The chief basis for the latter point of view, which has a very important bearing on the problem of pneumonia, is the work of Stillman, discussed in a previous report, by which it was shown that pneumococci of these types are found rarely in the mouths of the healthy, and then chiefly in the mouths of persons in immediate contact with patients suffering from pneumonia due to the same type of pneumococcus.

Now, if pneumococci of these types do not have a wide distribution apart from disease, the importance of the mere presence of these organisms in the respiratory tract becomes emphasized.

The experimental studies of Blake and Cecil, however, indicate that even with pneumococci of these fixed types, infection does not occur unless the cocci gain access to the lower respiratory tract and that this does not occur unless some accessory factor or factors operate to break down a defensive mechanism which ordinarily prevents bacteria reaching the lower respiratory tract, the lung itself. The following studies which have been carried on by Dr. E. G. Stillman indicate that even this alone is not sufficient, but that probably still other factors must be operating before infection occurs. The following is a description of these experiments and the results obtained:

Mice and guinea pigs were placed in specially constructed metal chambers and sprayed with cultures of pneumococci, the virulence of which for the test animals had been previously proved by the ~~intra~~



peritoneal inoculation of other animals. Of the 198 mice used in these spraying experiments, 34 were killed and autopsied at intervals of from 1/2 to 48 hours after spraying. Pneumococci of the same type were recovered from the lungs of 19 mice autopsied within 1 hour of spraying. After this period the incidence of recovery of pneumococci from the lungs rapidly decreased. Of the remaining 164 mice which were sprayed and allowed to live, all survived for 10 days with the exception of 5 which died with a pneumococcus septicemia. These observations afford evidence that virulent pneumococci following inhalation may reach the lungs and may be implanted in the lungs in appreciable numbers but that in all but a relatively few instances the pneumococci are effectively disposed of without injury to the animals.

Of 40 mice sprayed with hemolytic streptococci 19 were killed. Hemolytic streptococci were recovered from their lungs regularly up to the 7th hour after exposure, in half the mice after the 8th and 12th hour but not after 24 hours. The heart's blood and spleen cultures were negative. Of the 21 mice allowed to live, only 9 survived 14 days, the remaining 57% dying of a hemolytic streptococcus septicemia on the 2nd to the 12th day.

In the case of 33 mice sprayed with non-virulent B. influenza the recovery of the organisms was much more irregular. B. influenzae was not recovered from either of 2 mice killed at the end of 1, 2, 4, 6, and 24 hours, respectively, after spraying, but was present in one animal after 70 hours. The heart's blood and spleen cultures remained sterile. All of the mice not killed survived the 10 day period of observation.

These experiments suggest that even in highly susceptible animals (mice) virulent pneumococci are apparently unable to multiply in

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the healthy lung tissues, and that some effectual mechanism exists for their rapid disposal when introduced by spraying.

This experimental work and also observation of the distribution and spread of the disease in man indicate the need for further study of the factors concerned in pneumococcus pulmonary infection other than the presence in the lung of the pneumococcus itself. It is possible that those factors may be entirely non-specific and may have nothing to do with a living virus. The other possibility, however, that even acute lobar pneumonia may be the manifestation of a secondary infection must always be kept in mind.

The discovery by Olitsky and Gates of a new variety of small bacteria, previously overlooked, in certain cases of influenza has led to the suggestion that those or similar organisms might be present in other types of acute respiratory diseases, even in lobar pneumonia itself. Studies bearing on this problem have been carried on by Dr. Avery and Dr. Morgan. In this investigation they were fortunate in having the support of Dr. Olitsky and Dr. Gates.

Some time was first spent in becoming familiar with the biologic characteristics of the strain of *Bacterium pneumosintes* originally isolated by Dr. Olitsky and Dr. Gates in the epidemic of influenza in 1918-19. The technique originally employed by them in isolating *B. pneumosintes* was used in making cultures from a series of cases of mild respiratory infections occurring during the past winter. Cultures were made both from filtered and unfiltered nasal secretions. There have been isolated 14 strains of bacteria having characters which serve to identify them with the group of organisms of which *B. pneumosintes* may represent the type; 7 were recovered from patients with mild respiratory infections such as "colds," tracheitis, bronchitis, etc.,

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4 strains were recovered from one patient with broncho-pneumonia, cultures being made at weekly intervals during the month of convalescence and the three other strains were obtained from patients in whom the clinical diagnosis of influenza was made. All the strains possess in common the following characteristics: they are small, Gram negative bacilli, are strictly anaerobic, and were cultivated directly from the filtrates of nose pharyngeal washings after passage through Berkfeld candles "V" - that is, they were filter-passing in the form in which they existed before artificial cultivation.

Variations in morphology and serologic reactions suggest that these strains differ in certain characters from *B. pneumosintes*. Further study of the cultural and immunological properties of these recently isolated organisms is necessary, however, before any certain opinions can be given as to their identity with or similarity to the strain of *B. pneumosintes* originally isolated. It is interesting that in several instances the bacteria were isolated from cultures made directly from filtered washings on blood agar plates. The possibility of chance contamination from kidney tissue, ascitic fluid, or animal passage was thus eliminated. Whatever relation these strains, and others that may subsequently be isolated, may bear to one another or to the original *B. pneumosintes*, and whatever may eventually be proved as to their causal relationship to acute respiratory disease, it has been demonstrated that bacteria of this hitherto unknown and unrecognized type are frequently present in the respiratory tract.

So far organisms of this type have not been isolated from patients suffering from acute lobar pneumonia. Nevertheless, the discovery of the incitants of the milder infections of the upper respiratory tract, which so often precede pneumonia, would undoubtedly go far in

solving the problem of the pathogenesis of pneumonia.

Studies along these and similar lines are in progress and will be pursued during the coming winter.

Growth Requirements of B. influenzae and other Bacteria.

In the last report there were discussed certain studies of Dr. Avery and Dr. Tjotta which had resulted in the discovery that the growth stimulating effect of blood on B. influenza was dependent on two separable and distinct factors, one in a vitamin-like substance, and the other a heat stable substance acting in extremely low concentration. They furthermore showed that both of these factors are found in certain plant tissues (potato and banana).

Dr. Avery and Dr. Morgan during the past year have greatly extended these observations. They have shown that yellow and white turnip, carrot, beet, parsnip, and sweet potato, when added to fluid media possess the same growth stimulating action as does white potato. Moreover, they have found that these vegetable tissues not only permit the cultivation of the so-called hemoglobinophilic organisms, but that they also greatly favor the growth of other entirely unrelated organisms. For instance, in the case of pneumococcus, not only is there a marked acceleration of growth in media containing fresh vegetables, but a seeding, too minute in itself to initiate growth in plain broth alone, will amply suffice to induce abundant multiplication in the same medium to which small pieces of sterile, unheated vegetable have been added. Moreover, in the plant tissue medium the zone of hydrogen ion concentration within which growth can be initiated is considerably extended beyond the limits of the range in ordinary bouillon. In addition certain other bacteria, which ordinarily fail to grow in the presence of free oxygen, multiply in a medium containing fresh plant tissue even though

no precautions are taken to exclude air. It is evident, therefore, that the presence in media of certain substances contained in fresh plant tissue not only supplies the necessary factors for growth of the hemoglobinophilic bacilli but furnishes the necessary requirements for the cultivation of other bacteria which ordinarily multiply only under certain restricted conditions.

The exact nature of the substances contained in plant tissue upon which these properties depend is not yet determined, but the studies so far made suggest that the growth inducing properties are related to the presence of certain oxidizing and reducing enzymes in fresh plant tissues as well as to the presence of so-called accessory food substances.

Chemical Basis of Methemoglobin Formation by Pneumococci.

Dr. Avery and Dr. Morgan.

One of the most striking and characteristic properties of pneumococci is the effect which they produce on hemoglobin, changing it into methemoglobin. This is made evident by the change in color which the medium undergoes when pneumococci are cultivated on blood medium. This phenomenon has formed the basis of considerable study in this Hospital in the past, as it was thought possible that it had a much deeper significance in connection with the problem of infection than is obvious at first sight. In studying this phenomenon several years ago Dr. Cole found that change from hemoglobin to methemoglobin occurred only when living organisms were present, and it seemed that the reaction was closely related to growth phenomena and could not be induced by extracts or filtrates of the organisms.

Avery and Cullen during later studies on enzymes of the pneumococcus made observations which indicated that under certain con-

ditions the transformation of hemoglobin into methemoglobin might be induced by extracts of pneumococci or filtrates of the cultures, no living organisms being present. In other words, it seemed that the reaction is probably due to a substance, probably a ferment, which might be separated from the bacterial cells.

During the past year Dr. Avery and Dr. Morgan have made still further observations which are highly suggestive that this phenomenon may be due to a simple inorganic substance formed in the culture medium during the growth of pneumococcus.

On testing certain pneumococcus culture filtrates for the power to produce methemoglobin, it was found that while these filtrates had little or no power to produce the reaction on laked blood, they very rapidly acted on crystalline hemoglobin in watery solution. This discovery was only made possible through the work of Dr. Heidelberger who had prepared a large amount of crystalline hemoglobin by a new method and primarily for another purpose. They were now able to study in detail the action of the filtrates of pneumococcus cultures on pneumococci and have found that free access of air to the culture during growth is essential for the reacting substance to be formed. They have found also that if reducing substances or catalase be present in the medium the formation of the reacting substance is entirely inhibited or more likely is destroyed as rapidly as it is formed. Further, it has been found that the substance first makes its appearance during the period of maximum growth of the organisms. This indicates that it is a product of cell metabolism rather than the resultant of autolysis. The reacting substance is thermolabile, being destroyed by exposure to the temperature of boiling water for 15 minutes. In a medium in which it has once formed it may persist for several days, the exact time de-

pending on the reaction of the culture fluid and the temperature at which it is preserved, being less stable in alkaline than acid solution and suffering progressive loss in potency with exposure to increasing temperatures.

In addition to these properties this substance gives certain chemical reactions which are indicative of its nature. For instance, filtrates of pneumococcus cultures containing this substance give a positive benzidin reaction which inactive filtrates fail to give. This test is based on the fact that in the presence of a catalytic agent, peroxidase, solutions of benzidin give a blue color on the addition of hydrogen peroxide. The benzidin test, therefore is recognized as a delicate method for the detection of hydrogen peroxide. It seems justifiable, therefore, to regard the benzidin reacting substance in pneumococcus filtrates as hydrogen peroxide, and since only filtrates giving this test produce methemoglobin it seems probable that the substance on which the latter reaction depends is hydrogen peroxide. This view is strengthened by the fact that a solution of hydrogen peroxide, even in great dilution, is capable of changing crystalline hemoglobin into methemoglobin.

Moreover, the same agents which are found to cause inactivation of the culture filtrates also render hydrogen peroxide inactive as regards methemoglobin formation. The fact that these agents are destroyed by catalase explains why culture filtrates have no action on whole blood since in whole blood catalase is always present.

Growth Inhibition by Pneumococcus Filtrates.

In a previous report it was pointed out that pneumococci fail to grow when reinoculated into filtrates of culture fluids in which they have previously grown. This phenomenon of growth inhibition was

found to be dependent in part at least upon the exhaustion of some nutritive substance in the medium. Experimental evidence indicated that the substance exhausted by the initial growth of the pneumococci and in the absence of which secondary growth can not be initiated, is probably of carbohydrate nature, since the addition of a readily fermentable sugar, even in small amount, to exhausted filtrates suffices to restore the growth supporting property.

The discovery that pneumococci produce peroxide during growth and that this compound can be readily demonstrated in culture fluids adds another factor which must be taken into consideration in dealing with this complex phenomenon. Hydrogen peroxide exerts a marked restraint upon growth of bacteria, and in sufficient concentration is definitely bacteriacidal. The presence of this substance, therefore, in culture fluids might itself explain, in some instances at least, the inability of organisms to grow in culture filtrates. It has, indeed, been found that *Staphylococcus aureus* cannot be made to grow in the filtrates of pneumococcus cultures which contain peroxide. Proof that the peroxide is in itself solely responsible for lack of growth under these conditions, is found in the fact that *Staphylococci* will grow in these same culture fluids after the peroxide has been destroyed by heat or lost by deterioration through age. Further, *Staphylococci* grow abundantly in culture filtrates of pneumococci in which the formation of peroxide has been prevented.

In the case of pneumococcus, however, growth inhibition of this organism in its own culture fluids is conditioned primarily by the exhaustion of fermentable substance, and only secondarily by the presence of the peroxide, since in culture filtrates in which peroxide is not present a second growth of pneumococci cannot be initiated unless



additional carbohydrate is added.

Production of Peroxide by Bacteria Other than Pneumococci.

The discovery of peroxide formation by pneumococcus has led to the study of the occurrence of this compound in the culture fluids of other cocci. It has been found that under similar conditions of growth *Streptococcus viridans*, an organism closely related biologically to pneumococcus, produces peroxide actively and promptly, this substance being readily demonstrable in culture filtrates within the first 18 hours of growth. The production of peroxide by hemolytic streptococci, however, is less constant and its formation always delayed. Of 23 strains of *S. hemolyticus*, 8 failed to give the peroxide reaction at any time, while the remaining 15 strains gave a positive reaction only after the cultures had been incubated 86 to 110 hours. Only 2 strains of *Staphylococcus aureus* have been tested and both of these failed to form peroxide. It is interesting in this connection to observe that *Staphylococcus aureus* possesses a marked catalyase which is capable of splitting hydrogen peroxide. This fact may account for the absence of peroxide in culture fluids of this organism.

Chemical Nature of Soluble Specific Substance of Pneumococcus Origin.

Dr. Avery and Dr. Heidelberger.

The study of the soluble, specifically precipitable substance which is excreted in the urine of patients suffering from pneumonia is being continued. This active substance is derived from the infecting organism and passes the kidney unchanged in specificity and appears in the urine in a form which reacts only with antipneumococcus serum of the same type as that of the infecting pneumococcus. The study of this specifically reacting substance has shown that it is not protein in nature. It is stable in boiling solutions; is not dialyzable

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but it is precipitated from solution by acetone and alcohol. These properties are now being made use of in an attempt to isolate and purify the substance. The protein of the urine is removed by boiling with dilute acetic acid. The neutralized and concentrated filtrate is now poured into 10 volumes of acetone, the aqueous solution of the resulting precipitate is then dialyzed until salt-free, and reprecipitated first with acetone and then with absolute alcohol. The specific substance differs from many of the impurities accompanying it in not being precipitated by phosphotungstic acid or mercuric chloride and in being insoluble in hot glacial acetic acid. The purified preparations have been found to retain calcium very stubbornly, but are still highly specific after this is removed so that the presence of calcium does not appear to be essential. One purified sample which has been tested contains 6% nitrogen and practically no free amino nitrogen; but on hydrolysis yields about 50 per cent of the total nitrogen as amino nitrogen and 20 per cent as ammonia. About 1.5% of sulphur was also found, and if this is an actual part of the molecule it would indicate that the specific substance has a molecular weight of at least 2000.

A product with practically identical properties and analytical figures is obtained by dissolving washed pneumococci in anti-formin and purifying as in the case of urine. It has recently been found that a further inactive fraction can be split off by precipitating fractionally with acetone and it also appears that the active substance is not a polypeptide or proteose, as it is not precipitated by mercuric chloride in neutral solution.

The serologic type specificity of the reacting substance remains unimpaired by these chemical procedures. This permits the employment of a delicate biologic test to detect the presence of the active

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substance throughout the various chemical manipulations incident to the search for its structural constitution. The conditions seem excellent for determining the chemical nature of this substance with which the type specificity of pneumococcus is so intimately bound.

Oxygen Therapy in Pneumonia.

Brief attention was given in the last report to the question of the administration of oxygen to patients with pneumonia, and the construction of a special chamber to be used for this purpose was briefly commented upon. The designing and construction of this chamber was undertaken two years ago by Dr. Stadie. With his departure for New Haven the work was continued by Dr. Binger. Dr. Stadie's report of his work appeared in March, and although the problem is still under investigation by Dr. Binger, who is accumulating further data from the study of patients in the chamber, it may be appropriate now to discuss with some greater detail the questions involved and to describe a little more accurately the apparatus employed.

The employment of oxygen as a therapeutic agent is not new. Shortly after the discovery of oxygen, and particularly following the demonstration of the role of oxydation in life processes, the inhalation of oxygen as a therapeutic measure began to be employed. Ever since then it has been employed to a greater or less extent, the frequency and degree of its use varying in waves like that of fashion in dress. The rational of its use, however, has never been firmly established and the method of its employment has been a varied as would be expected under the existing conditions. It may fairly be said that a very few years ago the general opinion of physiologists was that the therapeutic use of oxygen was entirely irrational and unsupported by experimental evidence. Consequently, although oxygen continued to be

employed in certain conditions, its use became chiefly a death-bed measure and, unfortunately, in most cases a measure employed chiefly for the purpose of creating an impression of great endeavor.

During recent years, however, there has occurred a great increase in knowledge concerning the phenomena of oxydation and reduction in the tissues and concerning the rôle played by hemoglobin in the respiratory mechanism. This advance is due largely to the accurate quantitative work of Danish and English physiologists, and to the work of Van Slyke in this Hospital as well as other American investigators. These studies have again supplied theoretical support to oxygen therapy; evidence has been accumulated to support the view that in cases where the blood passing through the lungs is unsaturated with oxygen it should be possible to increase the degree of saturation by increasing the percentage of oxygen in the alveolar air. Whether this would be practically possible in cases of infection of the lung could only be determined by the direct determination in these cases of the degree of saturation of the arterial blood after it passes the lungs.

Now, the work of Dr. Stadie has made the direct investigation of the arterial blood possible, so that it has seemed important that the question of oxygen therapy should again be investigated, in the hope of getting evidence, based both on theoretical considerations and practical experience, to justify its employment, especially in the treatment of pneumonia, but also in the treatment of cardiac and other diseases in which there is apparently a deficient oxygenation of the blood in the lungs. To make this study of any real and lasting value it was obvious that the administration of the oxygen should be made under conditions where the amount could be accurately controlled, where the discomfort to the patient should be reduced to a minimum, and where the investigation

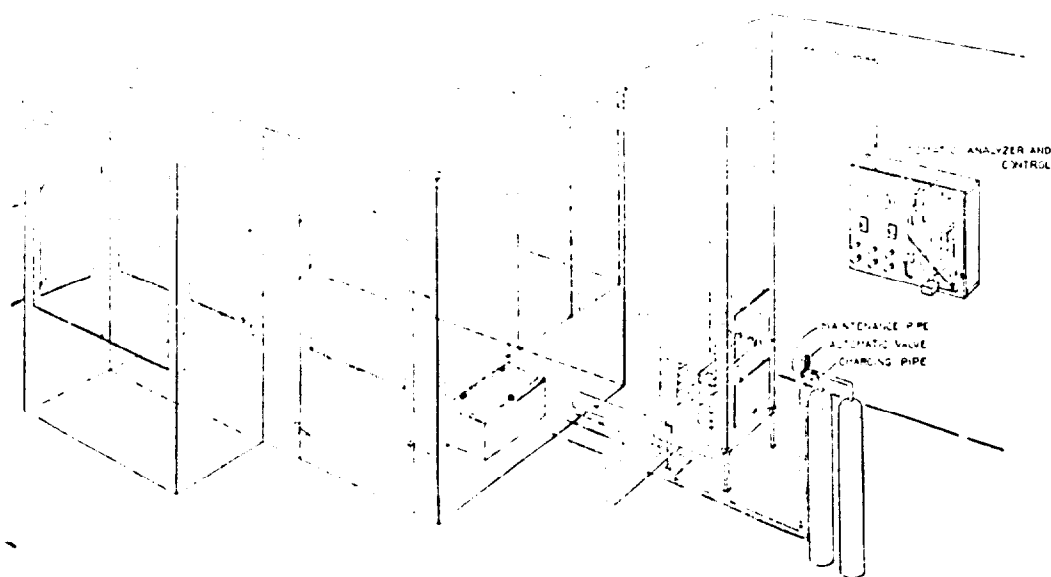
of the patient as regards analysis of the blood gases, studies of the respiratory movements, etc. should not be interfered with.

The older methods of administering oxygen were very unsatisfactory. When oxygen is allowed to flow through a funnel held over the patient's face and he thus breathes a mixture of air and oxygen of unknown amount, it has been found that diffusion of oxygen occurs so rapidly that the oxygen content of air in the lungs is but little influenced, or influenced in a very inconstant and irregular manner. The use of masks held tightly over the patient's mouth and nose causes so much discomfort and is so distressing that it can be continued only over very short periods of time.

After due consideration it became evident that to solve the problem whether or not the inhalation of oxygen is beneficial and then to determine the optimum amounts to employ, it would be best to construct a chamber in which the atmosphere could be regulated at will, and large enough to contain the patient and the necessary apparatus for investigation, and also sufficiently large so that the nursing within the chamber could be as effectively done as though the patient were in the open ward.

A chamber of this kind has now been built in one of the wards of the Hospital and after many changes and much effort on the part of Dr. Stadie, and later Dr. Binger, it has been made very satisfactory so that patients may be kept in it for days, or even weeks, in an atmosphere containing 50% of oxygen or even more.

A picture and diagram which give some idea of the construction of this chamber are shown on the following page:



TEXT-FIG. 1. Schematic drawing of oxygen chamber with ventilating system, carbon dioxide scrubber, cooling device, automatic oxygen analyzer, and filling and maintenance devices.



The photographic diagram indicates the construction of the chamber. While the chamber is not absolutely air tight, it has been so constructed by means of felt gaskets on the doors, etc. that the leakage is very little. There are two doors, one leading directly into the chamber for the admission of the bed, and a second one leading from the vestibule or lock. The nurse or physician enters the tightly closing lock closet, quickly closes the door behind him and then through a second tightly closing door enters the chamber. In this way loss of large amounts of oxygen is avoided. A food lock is provided so that food and other small articles may be taken in or removed without great loss by diffusion. The oxygen is delivered from tanks to openings near the floor of the chamber. The air is drawn out from the roof by means of a fan and is then passed over refrigeration coils and readmitted near the floor, a constant circulation of air being kept up. The diagram shows the air passing through a scrubber, that is a case containing soda lime, inserted for the purpose of removing the excess of  $C O_2$ . It has been found, however, much more satisfactory to have the scrubber directly in the chamber and to employ a second small fan for the purpose of forcing the air through it. A very satisfactory variety of soda lime has now been obtained which is comparatively inexpensive and with which the  $C O_2$  content of the chamber can be kept under 5%, even for very prolonged periods. While the automatic analyzer devised by Dr. Stadie was a most ingenious invention it has been found to require much attention, and a very simple method of analyzing the air has been arranged by Dr. Binger so that the nurse can take samples of air as often as required and within a few minutes determine the  $O_2$  and  $C O_2$  content. With a very little attention and care it has been found possible for the nurse to control the admission of oxygen so that the percent of oxygen in the air within

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the chamber is kept at an almost uniform level.

While in the first experiments that were made it was found necessary to use large amounts of oxygen, the loss by leakage, etc. has now been so controlled that the chamber can be properly employed at a cost of only \$4.00 per day for oxygen.

With this chamber and with the methods previously mentioned it is now possible to determine before the patient is placed in the chamber exactly the degree of oxygen saturation of his arterial blood, and then, after a patient has breathed an atmosphere of known composition for a given length of time, to again determine the degree of oxygen<sup>sat</sup> saturation and so to know the effect produced by the atmosphere of high concentration of known degree.

A number of patients have now been treated in this chamber for varying lengths of time, one patient was kept in the chamber almost continuously for thirteen days. One conclusion can now be drawn from these studies and that is that in most cases the anoxemia, that is, the oxygen saturation of the blood, may be relieved. Whether, however, this is of any real importance in the outcome of the disease only much more practical experience will tell. The impression so far gained, however, is that the method is of clinical value, that patients treated in this way are improved and their chances for recovery become greater. It seems off-hand that this should be the case and that the relief of this condition of anoxemia, which is certainly abnormal, should aid the patient in recovering. The mechanism of this relief, however, is difficult to know. Certainly the patient with pneumonia is not suffering from a deficient amount of oxygen supply to the tissue. In the case of anoxia of moderate grades, for instance, the amount of oxygen supplied to the tissues may be much less than in the case of pneumonia and still no



symptoms be manifest. As Stadie has pointed out, however, the tension at which the oxygen is delivered to the tissue may be very different in the two instances. These and relative matters must be studied before a clear idea can be had of the value of this form of therapy. The facilities for study of the problem in the Hospital are now excellent and it is planned to continue this work during the coming winter. If oxygen therapy proves to be useful, in order to make the method generally available it will probably be necessary to devise some simpler arrangement than the chamber. It may be possible to construct a rubber tent, to be placed over the bed, which will be effective. Dr. Binger is now at work designing an arrangement of this kind. For hospitals, however, a chamber such as built here will probably always be the most satisfactory.

Expedition to the Andes to Study the Physiologic Effect of High Altitudes.

In November, 1921, Dr. Binger was granted leave of absence to join an expedition to the Andes, which had for its object the study of problems closely related to those involved in the question of oxygen therapy. This expedition, financed by grants from the Royal Society and other institutions and by a grant from the Rockefeller Institute, was headed by Joseph Barcroft, F.R.S. C.B.E. the physiologist at Kings College, Cambridge, who is known chiefly for his contributions on the Respiratory Function of the Blood. The four Americans besides Dr. Binger were Drs. Bock, Forbes and Redfield of Harvard University Medical School, and Dr. Harrop of the Presbyterian Hospital. These men were joined in Lima, Peru, by Mr. Barcroft and Dr. Daggart of Kings College, and Professor Meakins of the University of Edinburgh. Through the courtesy of the Central Railroad of Peru, a British corporation, a baggage car was obtained which with a little carpentry was converted

into a splendid physiologic laboratory, and a freight car which served as a store for supplies.

After a little more than a week at sea level, while the laboratory car was being equipped and preliminary observations were being made, the party proceeded upward on the famous Andean railroad to an altitude of from 14,000 to 15,000 feet. The terminus of one branch of the railroad is at Cerro de Pasco, an ancient Peruvian town of some 9,000 inhabitants. Here the expedition made its headquarters and carried out the investigations planned at sea level. Cerro de Pasco is the site of the great copper mines, originally worked by the Incas and Spanish Colonials and in recent times chiefly by American interests. The officers of the Cerro de Pasco Copper Company were most hospitable and cooperative and, indeed, took a great interest in the work of the expedition which was not without its practical bearing. Throughout their sojourn on the mountain top the personnel of the expedition lived as guests of the Cerro de Pasco Copper Company in the Company Hotel.

The rapid transition from sea level to an elevation of 15,000 feet was not too well borne by the members of the party. Indeed all of them in greater or less degree succumbed to the condition known locally as "serroche", which is the Peruvian name for mountain sickness. This is a rather distressing condition, characterized by intense headache, nausea and vomiting, sleeplessness, shortness of breath, rapid heart rate and often fever and visual disturbances. Some of the party were incapacitated for a day or two, others for a week. Mountain sickness is now known to be due wholly to the low oxygen pressure in the atmospheric air found at high altitudes. It was the acclimitization to this diminished oxygen supply which the expedition undertook to study.

The subject has been under investigation for more than

half a century. Physiologists of many lands have made balloon ascensions, climbed to the tops of mountains and lived in steel chambers under diminished atmospheric pressure to study the consequent bodily changes. One of the most noteworthy expeditions was that headed by Prof. Haldane of Oxford to the summit of Pikes Peak, in 1913. This group of investigators found what Haldane had previously believed, that under conditions of oxygen want, the lungs had the ability to secrete oxygen just as the mucous membrane of the stomach secretes hydrochloric acid, and in this manner the normal pressure or concentration of  $O_2$  in the circulating blood is kept up, in spite of the reduced pressure of  $O_2$  in the atmospheric air. Recently new methods of investigation have been devised which have justified a reinvestigation of this problem. It is now possible to take samples of arterial blood and analyze them for oxygen and thus ascertain to what extent the arterial blood is saturated with oxygen. Knowing its capacity for oxygen and its content, we can express quantitatively the per cent of saturation. Mr. Barcroft used this technique on himself in 1920, when for six days and under conditions of considerable discomfort and, indeed, hardship, he lived in a steel chamber under greatly diminished atmospheric pressure. The result of his investigation was in direct contradiction to that of the Pikes Peak Expedition. No evidence of oxygen secretion was found, the arterial blood being only partly saturated with oxygen and containing no more than could be accounted for by simple diffusion of atmospheric oxygen through the lung tissue into the blood stream.

Haldane did not accept these results as conclusive, because of the relatively short exposure of the subject to low atmospheric pressure. And it was largely to settle this very important

controversy that Mr. Barcroft organized the Peruvian Expedition and selected for the work a place where humans had lived and had done hard physical labor for many generations at much diminished oxygen pressure, and at the same time a place accessible by rail and thus ideal from the point of view of transporting the necessary equipment.

The expedition divided its material for study into three groups (1) the natives, (2) the Anglo-Saxon resident engineers, some of whom had been at Cerro de Pasco for five years, (3) its own personnel. One of the first observations which it made was the marked and almost universal presence of cyanosis, - by which we mean a blue color of the skin, particularly of the cheeks, ears, nose, lips, finger nails. This we know to be due to an increase in the presence of reduced or unoxylized Hemoglobin - the red pigment in the blood that chemically binds and carries oxygen. Analysis of arterial blood taken from subjects of all three classes corroborated this observation, and showed, in fact, the presence of oxygen unsaturation to an extent wholly accountable for by the diminished partial pressure of oxygen in the air.

A great variety of other physiological phenomena, dealing with circulation and respiration, were inquired into. These are too numerous and technical to include in this report. One investigation which was of considerable practical interest to the Cerro de Pasco Copper Company was the study of the so-called "diffusion constant" of oxygen. A method of measuring the rate at which O<sub>2</sub> passes through the walls of the air sacs of the lung into the blood stream has been devised by M. Krogh. Employing this method it was found that those members of the Expedition in whom O<sub>2</sub> passed slowly were more seriously affected by high altitude than those in whom O<sub>2</sub> passed rapidly. This offers the possibility of determining in advance whether a given individual is or is not

adapted to life at high altitudes, and this is a matter of no little interest to the Cerro de Pasco Copper Company.

The expedition returned to New York in January, 1922. In brief the results were these:

- (1) No evidence of oxygen secretion could be found.
- (2) Oxygen unsaturation of the blood was present in all the subjects investigated.
- (3) Certain chemical changes in the blood were observed which could be interpreted as serving to increase the blood's affinity for O<sub>2</sub>.
- (4) A variety of anthropological measurements were made, indicating that the Andean has proportionately a larger chest than the Anglo-Saxon.

The results were presented by Mr. Barcroft at a series of lectures given at the Lowell Institute, in Boston, in February, 1922, and have since been presented by Dr. Binger and other members of the expedition before several medical and scientific societies. A complete report is about to appear in the Proceedings of the Royal Society.

Studies of Lung Volume.

Dr. Binger.

In a previous report mention has been made of a method for determining lung volume in patients who are suffering from shortness of breath. This method has involved numerous difficulties which have finally been surmounted so that it is now applicable to patients who are capable of very little co-operation.

By lung volume is meant, not the volume of lung tissue, but the volume of air enclosed in the air sacs and respiratory passages. The maximum amount of air which can be enclosed in the lungs on forced inspiration is known as the total capacity. Of this only a portion, - in normals, roughly, 72% - can be expired. That portion which can be expired

after forced inspiration is known as the "vital capacity", and that which cannot, but which remains in the lungs, is known as the "residual air". In normal subjects the vital capacity bears a definite relationship to certain other body measurements, such as height and weight and surface area, and, knowing these measurements, the vital capacity in a normal individual can be predicted to an accuracy of about 10%.

Employing these normal standards it has been found that in patients with heart disease the vital capacity is diminished and that the degree of reduction runs quite closely parallel to the severity of heart failure. Since this diminution in vital capacity is undoubtedly closely related to the phenomenon of dyspnea or shortness of breath so commonly seen in patients with heart disease it is of great importance that more should be learned of the nature and cause of this reduction. It is obviously important to learn whether the reduction in vital capacity involves a reduction of the total capacity or whether vital capacity alone is reduced. If vital capacity alone is reduced and the total capacity remains normal a proportionate increase in "residual air" must be present. Now, vital capacity can be measured with relative ease. The patient needs simply to take the deepest breath possible and then to blow into a movable tank, called a spirometer.

To measure the air remaining in the lung after forced expiration, or the residual air, is far more difficult. To determine this, indirect methods must be employed. Such a method has been devised by Dr. Binger and was described in some detail in the last annual report. The method has now been improved upon so that it can be easily applied in ill patients and it has now been employed in studying a number of patients suffering from pneumonia and heart disease. These studies have shown that in some cases of heart disease, apparently the

early ones, the residual air may be slightly increased. This lends support to the theory prevalent in the literature that the cardiac patient keeps his lungs in a more inflated position than the normal. In the more severely ill cardiac patients, however, especially those suffering from evidence of congestion in the pulmonary circulation, the residual air as well as the vital capacity have been found to be diminished.

That there is a close relationship between the air space in the lung and the condition of the pulmonary circulation is apparent. At present we have no methods for making direct observation on the lesser or pulmonary circulation which plays so large a role in the pathological-physiology of heart disease. The only approach we have to the state of the pulmonary circulation, besides the ordinary methods of physical examination, is through a study of the air content of the lung or lung volume. It is very probable where there is stagnation in the pulmonary circulation there is encroachment on the air sacs of the lung and diminution of the lung volume. A vicious circle is thus established, stagnation of blood gives rise to diminished air content and diminished air content in turn is followed by inadequate ventilation and imperfect gaseous diffusion from and into the blood. It is possible that forced deep breathing in the cardiac subject may lead to increase in lung volume and to decrease in the degree of stagnation in the pulmonary circulation.

The study of lung volume in heart disease may give important information concerning:

- (1) the mechanism of cardiac dyspnea;
- (2) the condition of the pulmonary circulation;
- (3) the problem of gaseous diffusion in the lung.

In pneumonia Dr. Binger has made a few preliminary observations on the question of lung volume. Here, as in the cardiac patient, the air sacs of the lung are encroached upon and even obliterated by congested capillaries and by the deposition of fibrin and exudate. As yet we have no very satisfactory correlation between the extent of pulmonary involvement and the course of the disease, particularly the appearance of such untoward symptoms as rapid and shallow breathing and cyanosis. It is hoped that a quantitative study from day to day in a pneumonia patient may help us find such correlation. It is knowledge of this sort that can give us the information necessary for rational oxygen therapy. Eventually we will need information about the diffusion of gases in the lung of the pneumonia patient and to get this information it is necessary to measure lung volume.

The method of measuring lung volume in pneumonia patients differs somewhat from that employed in the cardiac patients. We cannot get a satisfactory determination of the total capacity, because the individual sick with pneumonia is incapable of a forced deep inspiration and expiration. We must therefore measure the volume of the air in the lung during quiet, normal, breathing. For this purpose Dr. Binger has chosen an arbitrary though, to be sure, fairly constant point in the respiratory cycle, namely, the position at the end of quiet expiration. Lung volumes are measured at this point. At present Dr. Binger is engaged in the study of the resting expiratory position in a series of normal individuals for the purpose of establishing, if possible, some empirical constant whereby the measure can be related to some other physical factor, just as vital capacity is related to surface area.

It is hoped that having established this factor, we will know what the normal resting expiratory position should be, and then,



how much the patient under investigation deviates from it, and how he approaches it during convalescence.

The recent work of Krogh on the respiration of insects has shown at what point the respiratory tracts becomes sufficiently large to necessitate the respiratory motion. In the smaller insects simple diffusion of air into the tubules is sufficient to maintain the oxidative processes necessary for life. In large insects where the tubules are too long to permit diffusion alone to maintain the necessary oxygen concentration in the air sacs, respiratory motions are necessary. We have no way of measuring the size of the respiratory tract in man, except by measuring the volume of its air content. With this knowledge, and particularly with a knowledge of variations in size during diseased states, we may have valuable information on the facts controlling rate and depth of respiration.

Cyanosis.

Dr. Lundsgaard and Dr. Van Slyke.

Considerable attention has been given by Dr. Lundsgaard and Dr. Van Slyke to a consideration of the theoretical and practical aspects of cyanosis, basing their study on a historical review of the literature and on the recent quantitative blood gas estimations made here and by Dr. Lundsgaard in Copenhagen.

Cyanosis is the name given to a peculiar bluish color of the skin and mucous membranes seen in various abnormalities of the circulatory and respiratory systems. It has been known to be dependent on changes in the blood, particularly changes in the hemoglobin, and in most cases is related to an increased amount of reduced hemoglobin in the capillary blood. Dr. Lundsgaard, through quantitative studies, has shown that the occurrence of cyanosis is directly dependent on the absolute

amount of reduced hemoglobin present in the capillary blood, rather than on the ratio between it and the amount of oxyhemoglobin present. As a result of a series of estimations of the oxygen saturation of the arterial and venous blood in normal persons and in sick persons showing various degrees of cyanosis, he has determined that in general the presence of at least 5 grams of reduced hemoglobin per 100 c.c. of capillary blood is necessary before the skin acquires the bluish tint called cyanosis.

Now lack of normal saturation of the hemoglobin in the capillary blood may depend on one of several conditions, chief of which are, first, failure of the blood to undergo complete oxydation on passage through the lungs and, second, mixing of oxygenated and unoxygenated blood in the vessels, due to a shunting of part of the blood from the arterial to the venous side of the circulation, this part of the blood being unexposed to aeration in the lungs. Drs. Van Slyke and Lundsgaard have subjected the known facts concerning the latter condition to a mathematical treatment and have deduced formulae by means of which in any given case the fraction of blood which fails to pass through the lung can be estimated, if the degrees of unsaturation of the venous and of the arterial blood are known. By means of these formulae they have estimated that for cyanosis to become evident at least one third of the blood must pass through the shunt and be unexposed to aeration. This is much more than was previously thought to be necessary but the conclusions from the available data seem to be valid. In any case, their treatment of the subject has brought much clearness into a very complex and difficult problem and will undoubtedly be of great practical value in the rational application of oxygen therapy. Their paper dealing with the whole subject of cyanosis will shortly be published.

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Rheumatic Fever.

Dr. Swift.

Since the last annual report the clinical study of rheumatic fever by Drs. Swift, Boots and Miller has been facilitated by greater ease in securing suitable patients and by the cooperation of those previously admitted. It seems more and more probable that the disease often persists in these patients for years and that the relapses so frequently seen are due to a lighting up of latent infections. Thus rheumatic fever may be compared with syphilis or tuberculosis; diseases of known etiology, in which there are often long periods of latency, followed by relapses. The analogy may be carried still further: in tuberculosis we know that the most effective treatment is the building up of the patient's general resistance, and keeping him always generally fit. The same may be applied to the treatment of rheumatic fever. It is a disease accompanied by severe anemia and loss of weight; the rate of improvement usually parallels the recovery in weight curve; relapses often accompany a fall in this curve.

Rheumatic fever resembles syphilis in its tendency to relapse and in the manner some of the clinical manifestations respond to certain drugs. The lesions of tertiary syphilis usually disappear when the patient is given sufficient amounts of potassium iodid, even though this drug does not destroy the *Treponema pallidum*. Likewise, doses of mercury or salvarsan insufficient to eliminate completely the *Treponema* from the body of the syphilitic patient often arrest and remove secondary lesions.

The action of the so-called specific anti-rheumatic drugs - compounds of salicylic and phenyl-cinchonic acid - seem to us to be comparable to the remedies mentioned above. For nearly fifty years the

salicylates have been used successfully in combatting the fever and joint inflammation of patients with acute articular rheumatism, but the reason for this peculiar action is unknown. The lack of knowledge of the cause of this disease is partly responsible for the ignorance of the mode of action of the anti-rheumatic drugs. Our clinical studies have elicited evidence that even though patients under the influence of salicylates fail to show such signs of activity of the infection as fever, sweating, rapid loss of weight, and inflammation of the joints, there may be evidence of acute cardiac involvement, and the withdrawal of the drug is often promptly followed by relapse.

The problem immediately presents itself of determining which patients are still harboring a latent infection liable to become active as soon as salicylates are withdrawn. It has been found that most of these patients continue to have leucocyte counts of 10000 to 12000 or more. These moderate degrees of leucocytosis at times persist for months, and close clinical and electrocardiographic study of the patients indicate that they are probably still harboring the infectious agent. Practically all of the patients who have shown a normal number of leucocytes for several weeks have remained free from relapses. We now regard the leucocyte curve as a valuable guide in the administration of the anti-rheumatic drugs.

During the past year we have been studying the effect of treating rheumatic fever patients with compounds of phenylcinchonic acid. These drugs have the trade names of atophan, or cinchophen, and novatophan, neocinchophen or tolysin. They were originally used in the treatment of gout as it was found that they increased the excretion of urates by the kidneys. Later, it was found that they diminished the fever and relieved the arthritis of acute rheumatic fever patients in much the same manner as did the salicylates. It has been claimed that they are less toxic than

the salicylates. Our studies indicate that cinchophen is useful in relieving the distressing acute symptoms of rheumatic fever, but that it is too markedly a gastric irritant; however, neocinchophen does not have this distressing effect, seems less toxic than the salicylates, and in therapeutic doses is especially less irritating to the kidneys. This compound will probably not replace the salicylates, but is a valuable substitute in those cases where the patients show an idiosyncrasy to the older drug.

Drs. Boots and Cullen have completed their study of the reaction of joint fluids in the various forms of arthritis, and have shown that the reaction of the exudate in rheumatic fever is practically the same as that of the blood; while in those inflammations of joints due to invasion with hemolytic streptococci or staphylococci the reaction is acid. They have also shown that rabbits having an acute inflammation of the knees due to infection with non-hemolytic streptococci also have exudates in these joints acid in reaction. These findings point to the improbability of recovering streptococci from the joints of rheumatic fever patients, and also show that patients saturated with sodium salicylate could not have free salicylic acid in their joints.

Dr. Boots has made a microscopic study of the joint fluids of rheumatic fever patients, and found cell counts varying between 1000 and 32000; the higher counts occurred in the first days of inflammation when the predominating cells were polymorphonuclear leucocytes; later, large mononuclear cells were more in evidence.

He has also studied the nature of the joint involvement in patients with serum disease, and shown that small amounts of turbid fluid having from 5000 to 20000 cells per cubic millimeter can be aspirated from their joints. Both grossly and microscopically it is dif-

difficult, if not impossible, to distinguish this exudate from that found in rheumatic fever. The gross signs of joint involvement in serum disease are, however, much less marked. These findings show that a distinct inflammation of the joints may be excited by a foreign protein.

We have studied the action of sodium salicylate on the arthritis of rabbits infected with green streptococci. It has been claimed by some that these streptococci, of low virulence, are the cause of rheumatic fever, and that the lesions in rabbits infected with them are the same as those of rheumatic fever patients. We have been able to show that when rabbits were inoculated with green streptococci, after being put under the influence of sodium salicylate, they developed arthritis in practically the same proportion as non-salicylated rabbits; but that the non-salicylated animals had a considerably higher proportion of severely inflamed joints. Although the effect of sodium salicylate in these animals was different from that seen in patients, this is the first demonstration that this drug has a definite antibacterial action in the animal body.

Cardiac Disease.

Dr. Cohn and Dr. Levy.

During the past year, the work in this laboratory has been devoted to a continuance of the study of the action of quinidine both in patients suffering from auricular fibrillation and experimentally in dogs; to a continuance of the electrocardiographic study of the heart in rheumatic fever and to certain problems connected with the behavior of the normal electrocardiogram,

Last year we called attention to the discovery which had been made in Germany, that quinidine, a cinchona derivative, when given

to a certain percentage of persons suffering from auricular fibrillation, possessed the power of restoring the normal cardiac mechanism. At that time we examined experimentally in dogs, certain of its effects on the heart and circulation, describing especially an augmenting action on the contractile power of the ventricular muscle. Since then we have studied in detail the precise mechanism by means of which the drug is able to bring about a termination of the fibrillatory process.

We proceeded on the assumption that the theory of circular excitation, as the basis of the fibrillatory process was correct. That a process such as this could be made to exist was shown in this country in 1908 by A. G. Mayer. Later, in 1913, W. E. Garrey in America, and G. R. Mines in England suggested this process as the cause of fibrillation. Later still, in 1921, Lewis demonstrated the probable correctness of this view in elaborate experiments in dogs. In this theory, it is supposed that an impulse can course in a circular fashion in a piece of muscle, provided it is sufficiently large, especially if the muscle is in the form of a circle. The entrance of the great veins into the right auricle results in an arrangement of the muscle about them such that a circular strip of tissue is actually available, should there arise a stimulus ready to utilize it. The experimental evidence indicates that stimuli do arise which behave in this way. Now, in order that an impulse may travel round and round a circular strip of muscle, it is obvious that a given bit of tissue at a given point of the circumference must be ready to receive it, must be ready to be re-excited by it, when the impulse returns to its starting point. Other things being equal, the only practicable way a circus movement such as this can be stopped, is to place the muscle in a state in which, when the stimulus returns, it cannot receive it. This might be done by length-

ening the refractory period of the muscle, that period during which the muscle cannot be excited by the returning stimulus, or by rendering the rate of passage of the impulse so rapid that the impulse return before the refractory period had passed off. Whether these assumptions were the facts, we determined to put to the test of experiment.

For this purpose we employed anaesthetized dogs and studied with two galvanometers, the rate of passage of the impulse between two points on the auricular surface to which the galvanometers were connected. This we could do easily since we knew the distance between the two points, and, by photographing the shadows of the two galvanometer strings, knew the time interval between the appearance of the action current at these two points. The refractory period we learned by stimulating the auricle with induction shocks at various instants during diastole, and photographing the cardiac action current during this procedure. The calculations which were made of the time elapsing between the last contraction and the stimulus were then arranged chronologically. It was apparent that there resulted first a series of such periods in which no responses on the part of the auricles took place and then another series in which responses were obtained. The refractory period is the longest period in the first group, the group of the no-responses. The experiments are laborious so that not many were performed.

Our results were of great interest. We found first that the rate of conduction was either unchanged or made more rapid; it was never slower. This is a result contrary to the one which is desirable for this purpose. We learned however that the increased speed was not so great as to render ineffectual the effect which was obtained on the refractory period. The effect on this we found to vary, but there were cases in which the increase was 50 to 100 per cent, quite enough to render



a point on the circle of tissue refractory when the circularly travelling impulse returned to it.

We were able to show then that we possessed a means for altering a function of the heart muscle in a direction, such that the continuance of the circus movement which is at the basis of the fibrillatory process is rendered impossible. This favorable result is not uniform. That it is not, depends it appears on differences from animal to animal in the tone of the cardiac nerves. This inference we think is justified, for in a parallel series of experiments performed by Lewis, the influence of the nerves was excluded and in his experiments the results were uniform. The two series of experiments are complementary; they indicate the essential nature of the action of quinidine; they show why in patients that action is sometimes prevented.

During the year we continued in cases of rheumatic fever to collect information on the behavior of the heart from day to day. To the grosser forms of irregular heart action, such as heart block, we have already called attention; they were well known. We found also that minor changes in the coordinated contraction of the auricles and ventricles were to be detected. In certain cases it was indeed possible to show that with each recurring bout of fever, a disturbance of minor grade took place in the heart, even when no arresting manifestation could be demonstrated in the joints or elsewhere. We believe we possess evidence of a more certain nature than hitherto, of the slight but continuous ravages of the disease. How slight the changes are which may be reckoned as manifestations of disease we do not know. We have for this as well as for other reasons returned to a study of the normal electrocardiogram.

The normal electrocardiogram, we were taught, was a physical measurement so exact and so constant that it was placed in a class with the finger print method, as a means of identifying individuals. It

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was known that in certain persons, the position of the body and the phases of respiration influenced the form of the curves. In most instances these influences were unimportant, for curves could be made in a manner calculated to exclude their effect. Although these variations were small, they might be sufficient to render the interpretations we were putting on curves taken from rheumatic fever patients doubtful. We determined therefore to take series of curves for ten nearly consecutive days from a number of normal young individuals. The measurement of these curves is not yet complete. They are being measured not only with respect to the height of the waves, but also with respect to their duration and the time intervals between them. Great care was taken in making these curves. The individuals were always in the same position. We took care to know the extent and rate of their breathing. We did this by asking them to breathe from a spirometer and by having the spirometer connected with a signal magnet in such a way that each alteration of 100 cc. in the position of the spirometer was communicated to the signal hung before the electrocardiographic camera; the motions of the signal were photographed. In addition the inspiratory and expiratory motions of the spirometer were conveyed to a closed system of piston recorders. The lever of one of these also hung before the camera and was likewise photographed. The electrocardiograms themselves were made simultaneously with three galvanometers of the three usual leads. From curves like these, the direction of potential could be calculated without fear of error by the method of Einthoven, Bergansius and Bijtel. We believe that we have obtained sets of curves having an exactness not hitherto attained. It is clear even from an inspection of them and without measurement, that the curves vary from day to day to an extent not previously believed to be likely. We learn from this that during the course of disease, alteration in form of a grade disclosed in these series of normal

ones, are not to be given too great significance. We learn also that in a normal series, the time relations between the waves are constant to such an extent as to permit the inference to be drawn that a pathological process is present when there are variations as great as those which we found in the curves taken of rheumatic fever patients. We have a sound basis then for considering the behavior of the curves of the patients as evidence of a pathological process.

Having gone so far in the elaboration of our technique we determined to apply it in another direction. It is well known that when the heart enlarges, it gives rise to alterations in the electrocardiogram; the curve takes on one form when the left ventricle is hypertrophic, as in aortic insufficiency; it takes on an opposite form when the right ventricle is principally involved, as in mitral stenosis. These electrical signs have been useful in the clinic, but they have in our experience been on occasion misleading, for we have obtained curves resembling those found in enlarged hearts, in young soldiers and in civilians who, we were quite certain, were not the subject of disease. It occurred to us that the position of the heart in the chest might be an important contributing factor in producing the abnormal electrocardiograms. That small changes in the curves are possible as the result of respiratory movement, we have already mentioned. An exaggerated instance occurs in dextrocardia; here the heart is turned to the right rather than the left side of the body and the electrocardiogram assumes the appearance of a mirrored image of the usual curve. Having these experiments to guide us we planned to discover in a systematic manner precisely what was the effect of the position of the heart in the chest. Obviously we could not rotate the heart in the chest, but we could rotate the chest about the heart. Our method was as follows: Instead of taking leads from the limbs

(right and left arms and left leg) in the usual way, we took them from the apices of the largest equilateral triangle which we could apply to the chest, the base of the triangle stretching between the shoulders, the apex below the sternum. We assured ourselves that the curves taken from these leads resembled accurately those from the limb leads. We proceeded then to rotate this triangle through successive arcs of  $40^\circ$ , taking curves at each new position from those points of the chest wall to which the apices of the triangle pointed. By this means, we did in effect rotate the chest about the heart. In normal individuals, by quite small changes in the position of the triangle of leads, by changes quite possible in an anatomical sense, we obtained curves which actually resembled curves found in disease and resembled closely those often thought to be abnormal found in the young soldiers and civilians already mentioned. These were curves like those seen when the left side of the heart is large; in other positions, though anatomically quite improbable, we obtained curves resembling enlargement of the right side of the heart.

But this is not all. We turned next to patients whose hearts were known beyond doubt to be diseased and whose curves were characteristic of this condition. In these patients just as in the normal persons we applied the technique just described. We found that at some position in the course of rotating the leads, curves were obtained which resembled normal curves. In these cases, that curves of approximately normal appearance can be obtained is of theoretical interest. But that in obviously normal persons, yielding abnormal curves, normal curves can be obtained by a slight alteration of the position of the heart in the chest, is a matter of genuine practical importance. It does not simplify the interpretation of electrocardiograms, but it does something

more important; it shows how the normal individual may be saved from being considered the subject of chronic heart disease because of an abnormality in the curve. It need scarcely be pointed out that this danger is not theoretical. We have not by these experiments solved the problem of the relation of hypertrophy of the heart to patent abnormality in the electrocardiogram, but we have shown systematically and clearly that, especially in doubtful cases, the position of the normal heart in the chest must be taken into account as a factor in the production of abnormal curves as well as the position of the abnormal heart in the chest in the production of normal ones.

Clinical Studies of Quinidine.

The importance of the study of quinidine is better understood when it is appreciated that fibrillation of the auricles is present in about 40 per cent of the cardiac irregularities. In many instances the presence of the disorder is in itself serious. A majority of these individuals complain of unpleasant symptoms which are directly related to the presence of the irregularity. In about half of the cases treated with quinidine (in our own series 44 per cent) it is possible to restore the normal cardiac rhythm.

In May 1921, a preliminary report was published dealing with the therapeutic effects of quinidine sulphate in cases of auricular fibrillation. This work has been continued during the past year; twenty-five patients have been closely followed over relatively long periods of time.

Before attempting to alter the heart rhythm, we have found it better to combat heart failure, if this is present by rest in bed and by giving digitalis. A satisfactory plan of treatment has been outlined in which, after preliminary small doses to test for the presence

of idiosyncrasy to members of the cinchona group, gradually increasing amounts of the drug were given over a five day period. It is rarely necessary to continue therapy after the fifth day, for if the normal rhythm has not ensued after five days of treatment, it will probably not result from further doses; or, if it should appear, will last only a short period of time.

We have made a special effort to learn what alterations in the cardiac mechanism follow the administration of this drug by means of electrocardiograms made at frequent intervals. The first effect was usually acceleration of the ventricular rate. Occasional ventricular premature beats were also not uncommonly observed. Restoration of the normal mechanism occurred for the most part on the second, third or fourth day of treatment. The average dose was 2.6 gms. given in a three day period. The usual order of change in rhythm from auricular fibrillation to the normal mechanism is: retardation of auricular rate, that is to say the rate of the circular stimulus, impure auricular flutter, flutter, and then the normal rhythm. The actual transition from flutter to the normal sinus mechanism has once been photographed.

Certain untoward effects have been observed, both in this hospital and elsewhere. These accidents may be grouped under six heads: (1) unpleasant symptoms due to general effects of the drug, (2) sudden, though not fatal collapse, (3) embolism, (4) sudden death, (5) occurrence of heart rhythms indicating that the heart muscle has been intoxicated, (6) induction of heart failure.

Patients with large hearts in whom multiple valve lesions exist have, in our experience, been unfavorable subjects for quinidine therapy. In them, extensive disease of the heart muscle may be presumed to exist. In these individuals unpleasant symptoms follow ingestion of

the drug. One patient died suddenly shortly after the resumption of the normal rhythm; autopsy failed to reveal the cause of death, nor is the mechanism of death in cases like this at all clear. If fibrillation of the auricles has been of short duration a favorable issue of the treatment is more likely. We are inclined to regard the difference in result as dependent on the slighter extent of cardiac injury, as this is indicated clinically by the size of the heart.

Attention has been called to the occurrence of ventricular tachycardia after quinidine. This abnormal mechanism assumes great clinical significance if it is borne in mind that in dogs injected with digitalis or strophanthin it is the immediate precursor of ventricular fibrillation and death. Ventricular tachycardia has in point of fact been observed five times in our patients; but giving quinidine has been promptly discontinued and all the patients have recovered.

The duration of the normal rhythm after a single course of therapy is variable, ranging from a few hours to six months. In a number of patients, small daily or weekly doses have served to maintain the normal rhythm for as long as a year. No cumulative effects need be feared, as the drug is rapidly eliminated.

Studies of heart size, vital capacity, blood pressure and venous pressure have also been made. These have as yet not been critically analyzed.

At present it is difficult to select from the various cases of auricular fibrillation which present themselves those which do well with quinidine. In view of the possibility of the appearance of toxic symptoms, the treatment should be carried out with the patient in bed, preferably in a hospital where the behavior of the heart may be studied with the aid of graphic records. Carefully administered, this

drug is a therapeutic agent of great value; indiscriminately given, it may on occasion, be expected to cause disastrous effects.

A Tele-röntgenographic Study of the Size of the Heart in Pneumonia, with Consideration of the Effects of Digitalis Therapy.

Observations made last year have been continued and amplified. In brief, it has been shown that in a large percentage of cases of lobar pneumonia and in a somewhat smaller number of cases of bronchopneumonia, there is, in the course of the disease, a significant dilatation of the heart, with return to normal size during convalescence. The increase in the cardiac silhouette probably represents an attempt on the part of the heart to augment systolic output by increase in the length of its muscle fibres. According to the "law of the heart", as stated by Starling, such an increase is favorable up to a certain point. Whether that optimum is exceeded in pneumonia it is not possible to say, though signs of circulatory failure are conspicuous by their absence. Digitalis administration, early and in sufficient doses, apparently served, in a number of instances in which dilatation might have been anticipated, to prevent alteration in heart size.

It is now known, through our earlier experiments that digitalis, in therapeutic doses, augments myocardial contraction and increases ventricular volume output. For this reason it makes no difference whether the increase in the size of the heart is compensatory or deleterious. In either event the drug is of benefit. In the first instance it serves to aid the heart in its effort to maintain an adequate circulation during a period of stress; in the second instance it helps, at least in certain cases, in preventing dilatation of the cavities of the heart which in all probability is the result of severe toxemia.



Nephritis.Dr. Lundsgaard, Dr. Van Slyke, Dr. Stillman and Dr. Linder.

By thorough clinical investigation, blood analyses and functional tests, the clinical histories of patients have been followed with a view to obtaining as logical a classification of nephritis as may be possible and of treatment according to classification. Among the observations made in connection with the blood chemistry have been the repeated determination of the urea index employed in conjunction with urea feedings tests. This was done in the anticipation that a more definite estimate of the reserve powers of urea excretion may thus be made possible. In connection with this work Miss Hiller has elaborated a new method for the determination of urea in small quantities of blood. It is intended while these data are being accumulated by relatively short observation periods on a fairly large number of nephritics of all kinds, to attack, one at a time, certain specific problems that arise in connection with the study. For example, the abnormally high blood fat of patients of the so-called "nephrosis" type raises the question of the nature of the metabolic disturbance which causes lipemia. Absorbed fat is normally either burned or deposited in the body's fat depots so rapidly that it never rises in the blood to the heights found in these patients. The question is presented as to whether such patients fail to burn fat as rapidly as do normal individuals. Total metabolism experiments following fat feeding have been made, therefore, on some normal persons and on patients with nephritis accompanied by excessive lipemia.

Another specific question is whether in so-called hydremia there is really a dilution of the blood with increased total blood volume, or whether the low protein content of the blood is due to decrease in the

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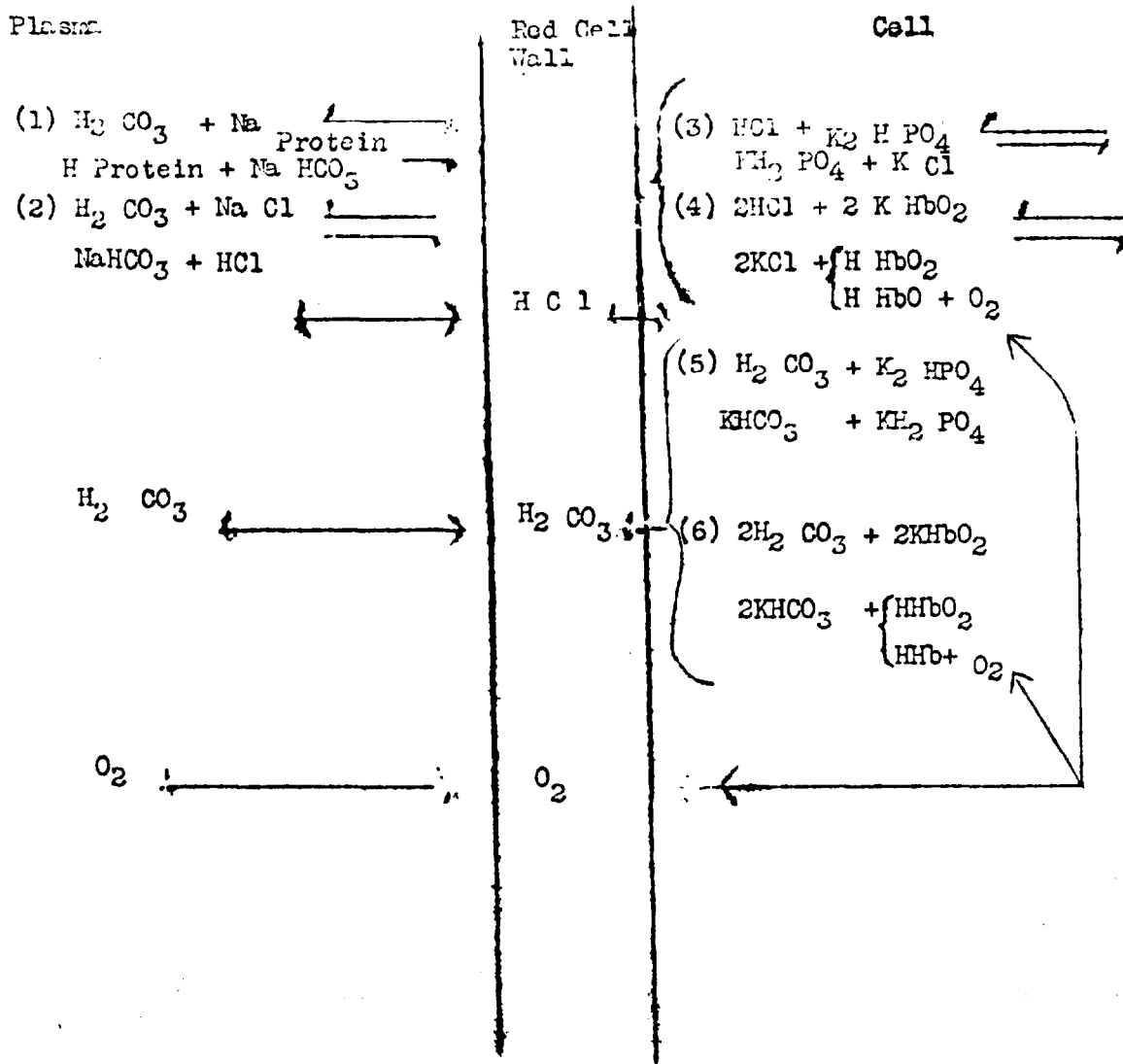
total blood protein content of the body without increase in blood volume. Dr. Linder has followed the variations in plasma proteins and has made repeated observations upon the blood volume in several of the cases. His results indicate a considerable degree of constancy in the plasma volume, in spite of marked changes in the plasma proteins, in the amount of edema and in the clinical state. It therefore appears probable that the "hy-dremia" of nephritis is not a condition of dilution of the blood, but depends upon a loss of protein from the plasma. The nature and quantity of the protein found in the urine is therefore engaging our attention.

Studies on Acid-Base Equilibrium in the Blood Carried on in the Chemical Laboratory Under the Direction of Dr. Van Slyke.

Drs. Austin, Cullen, Hastings, Heidelberger and Neill.

The study of diseases in which there is a disturbance in the acid-base equilibrium of the blood is dependent upon a knowledge of the quantitative relationships existing between the various substances which contribute to the maintenance of this equilibrium and presupposes information regarding the limits of variation to be found in normal individuals. It has been the purpose of the work done in this laboratory to advance our knowledge of the quantitative relationships involved, to determine the limits of variation to be found in normal variations and to apply the results thus obtained to the study of nephritis and pneumonia. Intimately concerned with this problem is the study of normal respiratory processes which is also under investigation.

The reactions involved in the respiratory changes of the blood may be qualitatively represented as follows:-



Increasing the  $H_2CO_3$  concentration in the tissues causes the formation of  $NaHCO_3$  in the plasma and  $KHCO_3$  in the cells. The resulting acidification of hemoglobin causes the partial liberation of oxygen. Conversely, increasing the  $O_2$  concentration in the lungs sends the reactions in the opposite direction and aids in the freeing of  $CO_2$ . The quantitative measurement of these reactions has received our attention during the past year.

In order to clarify the conception of buffer substances which play such an important role in biological fluids a theoretical

paper was written on the subject of buffer measurement, and the buffer values of weak acids, bases and amphoteric substances, of various dissociation constants. Despite the work that has been done with buffers, these relationships have not heretofore been worked out; in fact, there has not even been a unit for expressing buffer effect in qualitative numerical terms. As such a unit we have used the amount of strong alkali or acid required to cause unit change of pH. On this basis, a solution possesses a buffer value of 1 when the pH changes at the rate of 1 unit per gram equivalent of added strong acid or alkali. Mathematically expressed, the buffer value of a solution is  $\frac{dB}{d\text{pH}}$ , when dB is the increment of added strong base (NaOH or KOH), d pH the accompanying increment of pH. From the quantitative known relationships, formulated according to the mass law, of hydron concentration to the ratios  $\frac{\text{buffer salt}}{\text{free buffer acid}}$  and  $\frac{\text{buffer salt}}{\text{free buffer base}}$  (in the cases of weak acids and weak bases respectively

acting as buffers) it was possible to derive by means of the differential calculus a general equation expressing the buffer value of any buffer solution, viz.

$$\text{Buffer value } \frac{dB}{d\text{pH}} = 2.3 \left( \frac{KC}{(K + [H^+])^2} + [H] + [OH] \right)$$

For weak acids as buffers,  $K = K_a$ , the acid dissociation constant.

For weak bases  $K = \frac{10^{-14}}{K_b}$ , where  $K_b$  is the dissociation constant of the buffer base. For solutions near enough to neutrality so that  $[H^+]$  and  $[OH]$  are relatively negligible, viz. between pH 3 and pH 11. a zone covering nearly all biological solutions, the equation simplifies to

$$\text{Buffer value} = \frac{dB}{d\text{pH}} = \frac{2.3 KC [H^+]}{(K + [H^+])^2}$$

As is evident, the effectiveness of a given buffer is different for every  $[H^+]$ . The effectiveness of every buffer at varying pH follows the same curve, which reaches its maximum when  $K = [H^+]$  (or  $10^{\text{pH}} K = \text{pH}$ ), and becomes insignificant when the ratio  $\frac{K}{[H^+]}$  falls outside the limits  $\frac{100}{1}$  and  $\frac{1}{100}$ , that is, when  $-\log K$  differs from pH by more than 2 units. At its maximum effectiveness, when  $K = [H^+]$  any buffer is half in the form of free buffer acid or base, half in the form of its salt. At this point the value of an M/l solution (where  $C = 1$ ) of the buffer is  $\frac{2.3 K^2}{(2K)^2} = \frac{2.3}{4} = 0.575$ . The molecular concentration, and hence the molecular weight, of a dissolved buffer may be ascertained by dividing 0.575 by the observed maximum  $\frac{d B}{d \text{pH}}$  value. The latter is experimentally determined by adding known increments of strong base ( $d B$ ), or acid ( $-d B$ ) to the solution and measuring the pH change, ( $d \text{pH}$ ).

The  $[H^+]$  of maximum buffer value also indicates the dissociation constant of the buffer acid or base, since at this point  $[H^+] = K$ .

For buffers containing more than 1 buffer group (polyvalent weak acids or bases, or amphoteric substances) the buffer value is the sum of the buffer values of the different groups.

$$\frac{d B}{d \text{pH}} = 2.3 C [H^+] \left( \frac{K_1}{(K_1 + [H^+]_1)^2} + \frac{K_2}{(K_2 + [H^+]_2)^2} \right)$$

These principles were then applied to the study of hemoglobin and to the determination of the hitherto unknown second and third dissociation constants of citric acid.

The experimental work on these problems has been carried on by Austin, Cullen, Hastings, Heidelberger and Neill. For our experimental work it was first necessary to improve our methods of analysis and technique of handling blood and gases. With a new constant volume blood gas apparatus we were able to obtain results which had an average error of 1 part in 500. The modified Haldane apparatus for the analyses of gas mixtures has an error of similar magnitude.

It was further necessary to have at our disposal relatively large quantities of pure oxyhemoglobin. A method for obtaining this has been successfully developed by Dr. Heidelberger. The oxyhemoglobin is precipitated from water solution by saturation with a mixture of  $\text{CO}_2$  and  $\text{O}_2$  in the ratio of 4 to 1. It is then recrystallized several times by dissolving in alkali and repeating the precipitation process. Separation from electrolytes is finally effected by dialysis under pressure in collodion sacs. The hemoglobin thus obtained retains its full oxygen combining power and is practically free from electrolytes and other foreign substances.

With this material and technique we have obtained the data which is given below:

(1) The amount of alkali bound at pH 7.40 to a gram molecule of oxyhemoglobin is  $2.15 \pm 0.10$  equivalents.

(2) The amount of additional alkali which 1 molecule of reduced hemoglobin takes up when it is converted to oxyhemoglobin at pH 7.40 is  $0.68 \pm 0.10$  equivalents.

(3) The ratio of alkali combined with hemoglobin to the change in oxygenation, i.e.  $\frac{d B}{d H b O_2}$  is constant for all degrees of oxygenation.

This bears out L. J. Henderson's assumption.

(4) The molecular buffer value of oxyhemoglobin,  $p_o = 2.64$

The molecular buffer value of reduced hemoglobin,  $p_R = 2.45$ .

(This latter value is obtained indirectly and is subject to revision.)

These values are constant over the physiological range of pH.

Using these molecular buffer values and applying the principles laid down in the theoretical buffer paper written by Van Slyke it can be shown that at least 5 monovalent acid groups are involved in the reaction between alkali and hemoglobin over the physiological range.

(5) The relationships existing between oxyhemoglobin, reduced hemoglobin, base bound by hemoglobin and pH are summarized in the equation

$$B = 2.64 [\text{HbO}_2] (\text{pH} - 6.585) + 2.45 [\text{Hb}] (\text{pH} - 6.80)$$

(6) It is important that the above constants which are those for horse hemoglobin are different for dog hemoglobin.

Performing experiments on horse blood, similar to those made with hemoglobin, we have obtained the following results:

(1) The average buffer value of oxygenated blood from pH 7.2 to 7.5 was found to be 25.3 and for reduced blood 24.4.

(2) From pH 7.2 to 7.5 the buffer value of blood was constant, the degree of oxygenation being constant.

(3) The hemoglobin was found to be responsible for about 75% of the total buffer value of the oxygenated blood and about 73% of the buffer value of the reduced blood. The per cent of the buffer value attributable to the bicarbonate was 6.9 and 9.0 respectively. The loss in buffer value of hemoglobin when blood is reduced is thus only partially compensated by the increase in  $\text{BHCO}_3$ .

(4) The decrease in  $\text{BHCO}_3$  per molecular increase in oxygen, i.e. the  $\frac{dB}{d\text{HbO}_2}$  ratio varied from 0.50 to 0.59 at pH 7.30 and were con-

stant for all degrees of oxygenation. These values were lower than those found for pure hemoglobin solutions.

(5) With increasing pH the  $\frac{dB}{dHbO_2}$  ratio increased at the rate of 0.02 per 0.1 pH over the range 7.2 - 7.5. Assuming that only one acid group in the hemoglobin molecule is changed it will be possible with this data to estimate the dissociation constants of this group when reduced hemoglobin is changed to the oxygenated state.

A theoretical paper on "The Normal and Abnormal Variation of the Acid-Base Balance of the Blood" has been published. In it the demonstration has been made on the basis of facts found in this and other laboratories, that associated with each condition of high, normal or low blood bicarbonate may be a high, normal or low pH. There are thus 9 possible and actually observed combinations of  $BHCO_3$  and pH, of which only 1 is normal, viz. that in which both pH and  $BHCO_3$  are normal. Each of the other 8 conditions indicates a specific abnormality either in the metabolic processes by which non-volatile acids are formed and excreted, or in the respiratory processes by which the  $CO_2$  tension in the blood is regulated. For the general diagnosis of an abnormal condition, determination of both the pH and bicarbonate existing in the circulating blood becomes obviously necessary, although in special conditions abnormality in one factor is so characteristic, such as the low  $BHCO_3$  in diabetic acidosis, that the other can be neglected.

Since, if two of the three variables pH,  $BHCO_3$  and  $H_2CO_3$  are known, the other may be calculated and the acid base condition of the individual is determined, we have undertaken to determine the first two in normal individuals, in nephritis and in pneumonia patients. The method used for the total  $CO_2$  of the blood has already been described.



A colorimetric method for the determination of the pH which is simpler than electrometric and more accurate than previous colorimetric ones was devised by Cullen. The pH of plasma diluted 20 times under oil with neutral saline, without loss of CO<sub>2</sub>, determined colorimetrically at room temperature may by an empirical correction, which is constant for each species, be converted to the pH which would have obtained at 38°.

The question was recently raised by Evans, of England, as to whether electrometric pH estimations on CO<sub>2</sub>- containing solutions are valid. Evans claimed that some acid is formed by reduction of CO<sub>2</sub> at the electrodes, and causes the pH to be marked<sup>ly</sup> too low. Cullen and Hastings have checked the matter up carefully on phosphate solutions with and without CO<sub>2</sub>, and found theoretical results in both cases. They believe Evans could not have been sufficiently accurate in regulating the CO<sub>2</sub> tensions in his electrodes.

In connection with our nephritis service, the determination of the acid-base balance by combined pH and BHCO<sub>3</sub> estimations has already proved of clinical value. We are applying these principles and methods to the study of the acid-base balance in pneumonia. An experimental application has also been made to the acidosis produced by anesthesia.

Since the relation between the reaction, the CO<sub>2</sub> tension and the bicarbonate content is conveniently expressed by the Haas-elbalch equation

$$pH = pK' + \log \frac{BHCO_3}{\alpha CO_2}$$

it was thought necessary to redetermine the two constants pK' and  $\alpha$  with accuracy in order to make the equation clinically useful. We

wereable to confirm the value for  $\alpha$  , the solubility coefficient for  $\text{CO}_2$  in blood, as originally determined by Bohr. We found the value for  $\text{pK}'$  as originally determined by Hasselbach to be correct for plasma but incorrect for the whole blood or hemoglobin solutions.

#### Studies of Protein Chemistry.

##### Miss Hiller.

The investigation of the behavior of the different protein precipitants used in studying blood and protein digests have been completed. The precipitants studied include picric, trichloroacetic, metaphosphoric, and tungstic acids, colloidal iron, mercury salts, and alcò hol. The amino and peptide nitrogens were determined in the filtrates from blood with and without the addition of known amounts of amino acid and pepetone nitrogen. The action of the precipitants on Witte peptone solutions was also studied, in order to ascertain which ones remove most completely the intermediate protein products, and which remove the protein with a minimum of the intermediated. The data obtained will enable future investigators to choose precipitants adapted to their especial objects in a way that has not heretofore been possible. The immediate object of this work was to ascertain conditions for studying the absorption of intermediate products along with the amino acids during protein digestion. It is hoped to attack this problem, which is an unfinished portion of the study of the fate of protein digestion products.

RUFUS COLE