

REPORT OF THE DIRECTOR OF THE HOSPITAL

October 19, 1923

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Gentlemen:

During the year no noteworthy change in the personnel of the staff of the Hospital occurred but word has just been received of the appointment of Dr. Christen Lundsgaard as Professor of Medicine in the University of Copenhagen, one of the first academic posts in medicine in Europe, so that Dr. Lundsgaard will undoubtedly leave before long to assume his new duties. He first came to the Hospital in 1917 as a Fellow, remaining but one year. He returned in 1921 as Associate in Medicine and Resident Physician. On July first of this year he was advanced to the rank of Associate Member. During his connection with the Hospital Dr. Lundsgaard has carried on important researches in connection with the physiology and pathology of respiration and the success of this work here has undoubtedly been largely instrumental in causing him to be appointed to the very honorable position in Copenhagen.

His departure will leave a vacancy which should be filled as soon as possible. During the past decade there has occurred a great increase in interest in the study of diseases of disordered metabolism, and there are now many more men trained in the application of the science of chemistry to the study of diseases than were available when the Hospital was opened. Consequently a constantly increasing share

of the Hospital resources are being devoted to this kind of investigation. It is therefore important that a clinician with a thorough training in chemistry should be secured to complement and assist Dr. Van Slyke in the investigation of diseases metabolic in nature.

Of the Resident Staff, Dr. Boots and Dr. Dahl have resigned, and Dr. Crawford of Edinburgh and Dr. Andrews of London have received appointments as Assistant Resident Physicians.

It is gratifying to report that very substantial progress has already been made in the two new undertakings first mentioned in the last report. Especially important have been the results obtained by Dr. Rivers and Dr. Tillett in the investigation of chicken-pox. The details of this work are given in Dr. Rivers' report.

The division of the work in the Hospital into several parts, each carried on by a group of workers working more or less independently, has progressed and this is reflected in the present report which is chiefly composed of the reports of the heads of the various divisions.

#### Infectious Diseases of the Respiratory Tract.

The studies carried on have dealt chiefly with problems relating to pathogenesis and to problems relating to the biological properties of pneumococcus.

#### Pathogenesis of Lung Infections.

Dr. Stillman has continued the study mentioned in the last report concerning the disposition of pneumococci and streptococci which have reached the lung following spraying. The tentative results previously mentioned have been confirmed and the conclusions

published.

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In a continuation of this study it was found that if alcohol is administered to mice and the mice are then exposed to air containing pneumococcus in suspension, there occurs a delay in the disappearance from the lungs of the inspired bacteria. Although pneumococci will disappear from the lungs of normal mice following inhalation within four hours, in mice intoxicated by either inhalation of alcohol or by interperitoneal injection of alcohol, the period of removal is delayed in most instances until after 24 hours. Furthermore, whereas the blood stream is seldom invaded in normal mice following inhalation of pneumococci, about 40% of intoxicated mice develop a septicemia. In two instances pneumococci were found in the heart's blood of intoxicated mice one hour after inhalation.

Acute alcoholic intoxication also markedly increases the mortality of mice following spraying. Whereas only about 3% of normal mice die from a pneumococcus septicemia following spraying with pneumococci, mice intoxicated by interperitoneal injection of a 10% solution of alcohol or by inhalation of alcohol have a mortality of 30% and 50% respectively. In order to determine whether the administration of alcohol is favorable to the invasion of other bacteria, streptococcus hemolyticus and B. influenzae were also used. The effect of intoxication is not as definite where these organisms are employed in the experiment as when pneumococci are sprayed, probably because the bacteria mentioned are not removed as rapidly from the lungs of normal mice. However, even when these organisms are employed, septicemia is definitely more frequently encountered in the intoxicated mice.

Dr. Branch is making a histological study of the lungs of

the mice which have been sprayed with bacteria, with a view of obtaining information concerning the character of the earliest reaction which occurs when bacteria are implanted in the lung by inhalation.

Following the spraying of normal mice with pneumococci, in no case has a true pneumonia resulted. In some of the intoxicated mice, however, definite fibrinous pleurisy and marked congestion of the lung parenchyma have been found. In certain instances mice have been sprayed and then at a later date again sprayed either with or without previous intoxication with alcohol. In some of these mice the lungs have shown definite pneumonic patches, the sections having lesions resembling red and grey hepatization. This study is being continued with a view to studying the earliest pulmonary changes in the lungs following infection, and also to study the role of partial immunity in this process.

#### Virulence of Pneumococci.

Studies concerning the properties of pneumonia upon which virulence depend have been made by Dr. Cole and Dr. Dahl. It is generally believed that the properties of phagocytability and pathogenicity in pneumococci are directly related. An attempt is being made to determine whether this is accurately true and if so whether a phagocytic test can be devised that will permit measurements of pathogenicity.

Changes in pathogenicity brought about by various procedures are being investigated. Dr. Dahl has studied further the changes in agglutinability and pathogenicity, brought about by growth in immune serum (first studied by Miss Stryker) and also by growth in gradually increasing concentration of bile. By prolonged growth over many generations it has been found that bile fast strains may be obtained which

are avirulent and which do not become virulent even after many transfers through animals. This work is being continued.

Studies on the Biology of the Pneumococcus.

Two main lines of investigation have been followed: one dealing with certain physiological processes of the bacterial cell; the other with the immune-chemistry of the cell constituents.

Peroxide Formation by Bacteria and the Effect of Vegetable Oxidases on Bacterial Growth.

Dr. Avery and Dr. Morgan.

The studies have been the direct outcome of the earlier observations on bacterial nutrition. This work began with an analysis of the accessory substances in blood essential for growth of the so called hemophilic bacilli. This knowledge has brought out certain facts concerning the physiological activities of other groups of bacteria, particularly with reference to the relation between growth and certain oxidative reactions of the cell. In order to show the continuity of the work and to correlate the facts recently acquired with the observations previously reported, it seems desirable to review briefly the study as a whole.

Previous study of the growth requirements of B. influenzae has shown that the properties of blood upon which growth of this so called hemoglobinophilic organism depends are related to two separable and distinct substances. The combined action of both of these substances is essential, each being separably ineffective. On the basis of their relative resistance to heat these two factors can be differentiated one from the other. The so called "X" factor, which is associated with the pigment fraction of blood gives the peroxidase reaction and is destroyed by moist heat at 120°C.; while the second or so called "V" factor re-

sists boiling for short periods, but is destroyed by exposure to the higher temperature. Because of this difference in susceptibility to heat, blood can be deprived of the more labile substance (V) by autoclaving, and when so treated is no longer capable of supporting growth of B. influenzae. The X substance under these conditions, however, remains unaffected. This is proven by the fact that the addition of boiled extract of yeast (V) can activate autoclaved blood (X) and render it again suitable for growth. It is interesting in this connection to note as Hopkins, Meyerhof, and others have shown, that boiled extracts of plant and animal tissues, yeast and muscle, are of physiological importance in cell respiration. This V substance which is capable of complementing the X factor of blood has been found in extracts of green vegetables and yeast cells, and from the nature of its action it may be regarded either as a coenzyme or as a vitamin-like substance. The X factor, on the other hand, is found in hemoglobin and in certain of its derivatives (hemin) and is always associated with the fraction giving the peroxidase reaction with benzidine. Moreover, the X substance is active in such minute amounts as to suggest that it functions as a bio-catalyst.

Further study has confirmed the observation that the particular system represented by the combination of the X and V factors in blood has a complete analogue in the similar system of plant tissue. Unheated vegetable, for instance potato, possesses substances having the characteristics of both the X and V substances of blood. Therefore, as might be expected on the basis of this analogy, vegetable tissue can replace blood in the cultivation of B. influenzae.

The mechanism of the action of plant tissue in stimulating growth of the hemophilic bacteria is not wholly clear; analysis of the factors concerned, and the application of this knowledge to the growth of other bacteria suggests that this mechanism may be related to the physiological processes of cell respiration in a way similar to that of the substances described by Hopkins, Meyerhof, and others.

In discussing the growth stimulating action of plant tissue upon bacteria, it is of interest to correlate these facts with the effect of unheated vegetable tissue upon the growth of pneumococcus. It is, of course, well recognized that if a fluid medium is inoculated with an insufficient number of pneumococci little or no growth may occur, although the culture fluid may be optimal for growth if larger numbers of the organisms are introduced. However, if to this medium a piece of unheated potato is added, the minimum inoculum amply suffices to initiate prompt and abundant growth. Furthermore, the presence of plant tissue in the bouillon effects a remarkable alteration in the growth curve of pneumococcus. The period of lag is eliminated, the maximum rate of cell division is promptly initiated, and growth reaches its maximal development in the first eight hours of incubation. Moreover, sensitive as pneumococcus is to the initial reaction of the medium, the zone of optimal hydrogen ion concentration within which growth can be initiated is considerably extended both on the acid and alkaline sides in broth containing unheated vegetable tissue (potato). While the same mechanism which operates to facilitate growth of the hemophilic bacilli in plant tissue medium may be involved in the growth acceleration of pneumococci, in the latter instance another fact has been observed which may be of significance in explaining the action of these accessory factors. Whenever pneumococci are grown in bouillon



to which there is free access of air, hydrogen peroxide is formed in readily demonstrable amounts. This peroxide is toxic and accumulates in the culture fluid in concentrations which are bacteriocidal. On the other hand, under identical conditions of oxygen exposure, hydrogen peroxide is not demonstrable in cultures of pneumococci containing unheated plant tissue (potato). The absence of peroxide under these conditions is presumably due to the action of the catalase and peroxidase of the potato which possess the property of decomposing this substance. The presence of these enzymes, therefore, prevents the accumulation of hydrogen peroxide in the medium. The peroxidase in plant tissue is capable of acting upon the peroxide with the liberation of active oxygen which in turn can bring about further oxidative reactions. Plant tissue, therefore, may exercise the dual function of providing accessory growth factors, and of destroying deleterious growth products: peroxides.

Finally, plant tissue makes possible the aerobic cultivation of anaerobic bacteria. The mechanism of aerobic growth of anaerobic bacteria in media containing pieces of unheated vegetable seems to be related to the oxidation-reduction system present in plant tissue. The study of the formation of peroxide by pneumococci on exposure to air and of the absence of this product when the organisms are grown anaerobically suggested the following hypothesis which finds some justification in the facts presented. Although no experimental proof is yet available, it seems not unlikely that anaerobic organisms fail to grow in the presence of air not because atmospheric oxygen as such is a direct poison to the cell, but because of the toxic peroxides which are produced whenever the oxygen of the air combines with autoxidizable substances of the cell. This process of autoxidation gives rise finally to the formation of hydrogen peroxide,

which is known to be poisonous to the living protoplasm. Since these peroxides are formed only by the action of molecular oxygen on some autoxidizable substance of the cell, the absence of air precludes their formation. Under anaerobic conditions, therefore, the bacteria are not exposed to the injurious action of the products of autoxidation. If this assumption is correct, then the aerobic growth of obligate anaerobes in the presence of plant tissue finds partial explanation at least in the fact that the peroxide formed by autoxidation is rapidly broken up by the oxidizing enzymes of the plant tissue. Under these cultural conditions the sensitive cell is protected as effectively as if it were growing under anaerobic conditions.

Studies on Oxidation and Reduction by Pneumococcus.

Dr. Avery and Dr. Neill.

The fact that peroxide is formed and accumulates in readily demonstrable amounts in cultural fluids in which pneumococcus is growing, has led to a further study of certain oxidation-reduction phenomena of the pneumococcus cell. To obtain exact knowledge of these phenomena uncomplicated by the presence of cell growth, sterile extracts of pneumococci have been employed in these studies. In order to minimize the accumulation of oxidation products, the bacterial extracts were made from suspensions of anaerobically grown cells, care being taken to exclude the action of molecular oxygen during the processes of extraction. These sterile extracts, prepared by repeated freezing and thawing of pneumococci suspended in broth or phosphate solution, contain those active intracellular substances which are freed by disruption of the cell membrane, and which remain in the extraction fluid after prolonged centrifugation and Berkefeld filtration. Two types of cell extracts have been employed; one type prepared from

unwashed bacteria concentrated and extracted directly in small amounts of the supernatant broth in which they had previously grown; another type prepared from washed cells concentrated and extracted in phosphate solutions of known reaction and salt content. The sterile, cell-free extracts of unwashed pneumococci represent what may be termed a "complete" oxidation-reduction system. Extracts of this type are potent and exhibit the following reactions of oxidation and reduction: 1. rapid formation of peroxide upon exposure to molecular oxygen; 2. active reduction of methylene blue; 3. consumption of dissolved, molecular oxygen; 4. destruction (oxidation) of pneumococcus hemolysin; 5. destruction of oxyhemoglobin (methemoglobin formation.) On the other hand, the second type of cell extract, prepared from washed pneumococci suspended in phosphate solutions, is by itself wholly inactive, and owing to the absence of activating substances, is incapable of bringing about any of the oxidation reactions characteristic of the extracts of unwashed cells. The "incomplete" system of this type of extract, however, can be reactivated by the addition of boiled yeast extract, muscle infusion, or the cell washings. When complemented in this manner the extracts from washed bacteria also exhibit all the functions common to the potent extracts from unwashed cells. Both yeast extract and muscle infusion are known sources of autoxidizable substance, and it seems probable that these are the constituents concerned in the "completion" of the system. The actual source of the activating substances present in the cell washings had not been determined. It may be that activation in this instance is due to the presence of traces of broth, or of certain autoxidizable substances of cell itself which have been removed by washing.

In addition to other active intracellular agents, both types

of extract contain the concentrated endo-hemolysin described in 1914 by Dr. Cole. This hemolysin is exceedingly unstable and has been considered one of the most labile substances of pneumococcus origin. In view of the present work, it seems probable that in many instances this instability is due to oxidation. For the hemolysin in both types of extract has been found fairly stable if preserved under anaerobic conditions. However, if the type of extract containing the "complete" oxidation-reduction system is exposed to molecular oxygen, the hemolysin is very rapidly destroyed. On the other hand, the hemolysin may be exposed to the same oxygen tension in the "incomplete" or washed cell extract with comparatively little effect. In other words, molecular oxygen and constituents of the washed pneumococcus cell cannot oxidize the hemolysin molecule to non-hemolytic products in the absence of these autolizable substances which have been found essential in the other oxidation and reduction activities of sterile cell extracts.

The destruction of oxyhemoglobin (formation of methemoglobin), one of the most prominent characteristics of the living pneumococcus cell, is also an exceedingly active property of these sterile extracts. Quantitative measurements show that the hemoglobin destruction effected by the reduced sterile extract is much greater than that reported in earlier experiments in which no attempt was made to prevent oxidation of the pneumococcus cell preparations. In these studies it has been found that as much as 300 mg. of  $HbO_2$  may be destroyed by the addition of 0.5 cc. of the reduced extract to 4 cc. of blood. Here again, as in the case of hemolysin oxidation, autoxidizable substances seem to be required, as the constituents of the washed cell in the presence of molecular oxygen cannot convert oxyhemoglobin to methemoglobin. Throughout the entire investigation there have been evident many close analogies

between the systems in the sterile extracts responsible for peroxide production, methylene blue reduction, and oxygen consumption, and those responsible for the oxidation of hemolysin and the destruction of oxyhemoglobin (methemoglobin formation). In fact, it seems likely that all of these activities may be functions of the same, or quite similar systems, not only in the sterile cell extract, but in the living pneumococcus cell itself.

These results are now in manuscript form, to be published under the following titles: "Studies of Oxidation and Reduction by *Pneumococcus*":

- I. The production of peroxide by anaerobically grown pneumococcus cells under conditions not permitting active growth.
- II. The production of peroxide by sterile extracts of pneumococcus.
- III. The reduction of methylene blue by sterile extracts of pneumococcus.
- IV. The oxidation of hemolysin in sterile cell extracts of pneumococcus.
- V. The destruction of oxyhemoglobin (methemoglobin formation) by sterile extracts of pneumococcus.

The Soluble Specific Substance of *Pneumococcus*.

Dr. Heidelberger and Dr. Avery.

At the time of filing the last report, purification of the so called "soluble specific substance" of Type II *Pneumococcus* had been carried to a point at which the best preparation showed a nitrogen content of 2 per cent, yielded 49 per cent of reducing sugars when subjected to acid hydrolysis, and precipitated immune serum when present at a dilution as high as 1:2,500,000. The fact that the highest dilution reacting with immune serum also gave the Molisch test for carbohydrates, taken together with the increase in optical rotation and diminution of nitrogen content as successive preparation increased

in activity, was taken to indicate the probable carbohydrate nature of the soluble substance itself. Subsequent work has strengthened this viewpoint, and the data obtained have been published in the Journal of Experimental Medicine for July, 1923.

Refinements in the method of purification have led to preparations more active than those previously reported upon. Alcohol is substituted for acetone in the preparation of the concentrate from broth cultures of pneumococci, and the precipitate is centrifuged at high speed. A separation into three layers results. Of these the top and bottom layers contain so little active material that they can be discarded, and with them is eliminated in the very first precipitation, the greater part of the inactive substance in the broth. The middle layer, a gummy cake, contains the soluble specific substance, and is purified as already described. It has been found advantageous to add a final, third precipitation with solid ammonium sulfate.

A comparison is given of Preparations 17 and 21, which have not yet been reported. The former was obtained by the older method of purification, the latter by the improved process.

No.	Total N o/oo	S	P	C o/o	H o/o	Hydrolysis	Specific rotation. K <sub>D</sub>	Precipitation with immune serum
						Reducing sugars.		
17	1.2	None	Trace	46.2	6.1	79.0	+55.7°	1:3,000,000
21	0.46	-	-	46.8	6.0	70.5	+55.2°	1:12,000,000

It will thus be seen that in the more active preparation the percentage of nitrogen has sunk to scarcely more than a trace. The percentage of the reducing sugars found on hydrolysis did not, however,

show an increase, and this will be investigated further as it may be due either to failure to realize the optimum condition for the hydrolysis, or to the presence in the material of a non-polysaccharide portion, either as an impurity, or as a constituent of the active material itself.

The percentages of carbon and hydrogen found are not far from the theoretical values for a polysaccharide composed of hexose units, C, 44.4 per cent, H 6.2 per cent. That the hexose from which the greater part of the active substance in its present state of purity is built up is actually glucose has been definitely shown by the isolation of this sugar as the osazone from the products of hydrolysis of 0.4 gm. of Preparation 17. 0.063 gm. of purified osazone were obtained and identified as glucosazone both by the melting point and optical rotation.

While it thus appears that the soluble specific substance is actually a polysaccharide or glucoside, and not merely associated with a substance of this type, the matter is by no means proved, and three lines of investigation are now under way in the hope of deciding the question and thus rendering a true contribution to the chemical nature of bacterial specificity. These lines of work are:

1. Further attempts to purify the soluble substance by chemical and physical means.
2. Attempts to attack the carbohydrates by means of enzymes, molds, and bacteria, and investigation of the fate of the soluble substance under these circumstances.
3. Precipitation of the soluble substance with immune serum and its recovery from the precipitate.

A comparison of the soluble specific substance of Types II and III is now being made.

### Nature of the Alterations in Respiration in Pneumonia.

Dr. Binger and Dr. Brow.

#### The New Oxygen Chamber.

Building of the new Oxygen Chamber was not begun until the summer months - as it was not thought desirable to start work during the busy period of the Hospital year. The new chamber is now completed - except for a few minor details. It has not yet been tested, but should be entirely ready at the commencement of the pneumonia season. The chamber has been constructed with particular regard to safety from fire risk, tightness and simplicity and economy of operation. To secure all these factors a considerable initial outlay has been necessitated.

It is planned to use the chamber for the present for studying and treating cases of pneumonia. Of particular interest will be an investigation of external and internal respiration in response to oxygen therapy. It is planned to study the tension at which oxygen exists in the arterial blood as well as the oxygen unsaturation. The general scheme will be to continue to look for physiological evidence for the efficacy or lack of efficacy of oxygen in pneumonia, to learn the dosage required and the optimum time for administering the drug and to accumulate statistical evidence.

#### The Lung Volume in Lobar Pneumonia.

Dr. Binger and Dr. Brow have completed a study of alterations in lung volume in 10 cases of lobar pneumonia. The manuscript describing the work has been submitted for publication to the Journal of Experimental Medicine. They have measured the so called functional residual air in a series of normal individuals and in the pneumonia patients at different stages of the disease. This lung volume repre-



sents the volume of air enclosed in the lungs at the end of normal spontaneous unforced expiration. There were several reasons for studying this particular lung volume. First, it could be determined with no further cooperation on the part of sick patients than simply breathing through a rubber mouth piece with the nose clipped. Secondly, it obviated the adventitious and uncontrollable circumstances arising from pleuritic pain, muscular weakness and psychic state when the methods employed aim at determining the total lung capacity [vital capacity and residual air], this method requiring forced maximum inspiration or expiration. Thirdly, the functional residual air represents the volume of air actually functioning in respiratory exchange and as such must bear a close relationship to the area of pulmonary epithelium through which diffusion of gases occurs. It was soon learned that fluctuations in the volume of the functional residual air correspond to alterations in the size of the functionally active lung as shown by radiographic pictures and by physical signs, and those fluctuations may well therefore be interpreted as evidence of consolidation and resolution. For this reason the changes occurring at crisis were of especial interest. The general belief among clinical observers has been that crisis is not accompanied by immediate changes in the pulmonary lesion, or, at least, that there is no prompt "clearing up" of the consolidated area. Indeed, one of the striking characteristics of the change occurring at this period has been considered to be the great decrease in respiratory rate without any obvious change in the local lesion. It has been found, however, that drop in temperature, cardiac and respiratory rate is always accompanied by beginning increase in the volume of functional residual air. Furthermore, it has been observed that in those

cases in which fever, accelerated heart rate, and rapid breathing is sustained there is progressive decrease in the volume of the functional residual air. A close coincidence has been observed between these changes in symptomatology, lung volume, and the presence and disappearance of cyanosis. The relationship discovered is one of coincidence in time and cannot serve as an explanation of the mechanism of the symptoms involved, though it is conceivable that the characteristic rapid and shallow breathing in pneumonia results from the heightened metabolic rate of the febrile state, combined with a diminished diffusion area for gas exchange. That the breathing of these patients is actually rapid and shallow has been quantitatively established by many observations on respiratory rate, tidal air, and minute volume recorded by a graphic method. The result of an augmented rate and a diminished tidal air is usually an increase in the minute volume of pulmonary ventilation.

#### Acid Base Equilibrium in the Blood in Pneumonia.

This increase in minute volume which occurs may explain the low  $\text{CO}_2$  tension in the arterial blood of pneumonia patients during the febrile period which has been found to be present by Dr. Binger in collaboration with Doctors Hastings, Neill and Morgan.

In spite of the diminished tension of the  $\text{CO}_2$  in the arterial blood of febrile pneumonia patients, no true acidosis occurs in the sense of a change in pH 4. The hydrogen ion concentration is maintained at its normal level and so we cannot explain hyperventilation as the result of  $\text{H}^+$  ions on the respiratory center. Indeed a normal pH indicates a functionally efficient respiratory center.

This functional efficiency does not, however, signify the fact that the respiratory center is not approaching fatigue and ex-

haustion, nor that the organism does not eventually die from break down of the respiratory mechanism, by which we include both the central nervous and pulmonary apparatus.

Indeed, there is evidence which points to just such an eventuality. The work of Neuburg, Means and Porter reveals the fact that dogs with lobar pneumonia are unable to respond characteristically to the stimulus of  $\text{CO}_2$  after they have suffered from the dyspnea of experimental pneumonia. Such dyspnea can be prevented by sectioning the vagus nerves or blocking them with novocaine. Under these circumstances dyspnea does not supervene and the respiratory response to a  $\text{CO}_2$  stimulus is a normal one.

#### Nervous Mechanism of Dyspnea.

Dr. Binger and Dr. Brow are continuing studies in this direction and have started an inquiry into the nature of the vagal stimuli arising in the lungs, their origin and frequency, and the means of blocking them. The present working hypothesis is that lesions of the pulmonary capillaries are responsible for the stimuli.

They have conducted a series of preliminary experiments in dogs on the effect of acid injection into the lung before and after the vagotomy and a study of the resulting tachypnea. The first problem to be solved was to find a suitable anaesthetic - one which would keep the animal in a relaxed and insensitive state and still one which would not block the very impulses to which they were directing their attention. All the usual volatile and non-volatile anesthetics used for laboratory animals failed. They finally succeeded in achieving the necessary conditions by the use of Luminal given in small doses by stomach tube.

Further work on this problem is in progress and together

with the studies in the oxygen chamber will occupy the major portion of Dr. Binger's and Dr. Brow's time for the ensuing year.

Studies on Chicken-pox.

Dr. Rivers and Dr. Tillett.

In October of 1922 we began our study of the contagious diseases which occur most commonly in children. This study was undertaken in the hope of making observations which would add to the general knowledge of these diseases and which might eventually help in their prevention or cure. Chicken-pox is usually considered a mild disorder, but, nevertheless, for many reasons, it appeared to be a very suitable one on which to begin a study of this group of diseases.

The results of our year's work on the problem chosen for study will be given under five headings:-

- A.- Study of vaccinia in rabbits.
- B.- Clinical study of cases of chicken-pox in the Hospital.
- C.- Results of attempts to transmit chicken-pox to animals.
- D.- The effect of certain physical and chemical agents upon the virus recovered from varicella patients.
- E.- Study of the immunity produced by the virus recovered from varicella patients.

A.- Study of Vaccinia in Rabbits.

We began immediately to work with vaccinia in order to learn the proper methods of handling a virus which might be supposed to be somewhat similar to the one causing chicken-pox. This seems to have been a wise step as we feel that the success we have had in our work with varicella is due to the knowledge acquired in this way. We were able to acquaint ourselves with the methods of studying the lesions in the eye, skin and testicles when these organisms were inoculated with a virus. Furthermore, it was possible for us to confirm the

important observations of Calmette and Guerin and of Ohtawara. The former workers demonstrated that vaccine virus injected intravenously localizes in the irritated skin. The latter observer recovered vaccine virus from the blood of rabbits previously vaccinated on the skin, by injecting the blood into the testicles of normal rabbits and after 4 to 6 days removing the testicles, grinding them up, and testing for the presence of the virus by inoculating the testicular emulsion on the skin of other normal rabbits. These observations have a direct bearing upon our work in transmitting chicken-pox to rabbits and their importance will be seen in that part of our report.

#### B.- Clinical Study of Cases of Chicken-pox in the Hospital.

During the year 51 patients with chicken-pox were cared for in the Hospital. We encountered no instance of herpes zoster and varicella occurring in the same family at or about the same time. The blood picture was followed in most of the patients. In the severe cases a leukopenia was observed early in the disease but there was no marked secondary leukocytosis similar to that occurring in smallpox. The majority of the patients developed an eosinophilia during convalescence. No allergic response was demonstrated in patients recently recovered from varicella when fresh vesicle fluid was employed. Vesicle fluid was collected from lesions at different stages of development and a cytological study was made in fresh unstained specimens, in fresh specimens stained with different vital dyes and fat stains, and in specimens fixed and stained in many ways. Small pieces of skin were removed at various stages in the development of macules, papules and vesicles, fixed, stained and studied in various ways.

Certain clinical observations made during the year helped us

a great deal as they afforded indirect evidence that the virus is in the blood of chicken-pox patients and that the amount there does not necessarily correspond to the number of lesions in the skin. Furthermore, they focussed our attention upon the blood stream as a suitable place to recover the virus from patients. It was noticed that irritation seemed to have a direct influence on the localization of the virus in the skin, as evidenced by the appearance of an unusual number of vesicles at the site of irritation. The picture was particularly striking when the irritation involved the skin of the face and extremities, parts of the body usually least affected by the eruption of chicken-pox. This effect of irritation on the localization of the virus in the skin was observed in 5 of 51 patients.

C.- Results of Attempts to Transmit Chicken-pox to Animals.

At first our attempts to transmit varicella to animals were unsuccessful. We had no definite idea of how to proceed other than to inoculate different laboratory animals in various ways with material collected from as many sources as possible from chicken-pox patients. It is unnecessary to give the details of these negative results. Later, however, after our attention had been focussed upon the blood of patients as a source from which to recover the virus of varicella and after we found that vaccine virus can be demonstrated in the blood of vaccinated rabbits by injecting their blood into the testicles of normal rabbits, the work became more orderly and has yielded definite results. It might be well to give in detail the method employed and a description of the results obtained.

1. Method used in recovering a virus from varicella patients.

Blood was drawn from the patients usually during the first 24 hours after the appearance of the eruption. The blood was not ci-

trated and before clotting occurred was injected in 2 cc. amounts into each testicle of normal rabbits (2000 gm.). Large quantities of blood were used intentionally. At the time of inoculation the needle was moved around to produce a certain amount of trauma. Four days later the testicles were removed, ground up thoroughly with sterile, chemically clean sand and mixed with 10 cc. of physiological salt solution. The mixture was allowed to stand until the sand settled to the bottom. Strict asepsis was observed throughout the work. Portions of the testicular emulsion were tested for the presence of ordinary bacteria by means of cultures on blood agar, in broth and in Smith-Noguchi tubes. Other portions for future use were stored on ice either in the original state or after the addition of equal quantities of glycerine. Then 1 cc. of the emulsion was injected into each testicle of normal rabbits. Two places in the rabbits' skin were shaved and scarified. One of the areas was smeared with the emulsion, the other was used as a control. An eye of each rabbit was also inoculated. Both corneas were scarified with a cataract knife; one was inoculated with the testicular emulsion, the other was used as a control. The skin and cornea of each rabbit were inoculated to test for some visible reaction of a virus that might be present in the material injected into the testicles. The first few animals in each series showed very little reaction other than that which might be expected to follow the trauma of inoculation. The skin and corneas healed rapidly. The scrotum was edematous at times for 24 to 48 hours. The testicles when removed, were slightly swollen. Necrotic areas and often remains of the material injected studded the testicles in various places. This reaction was no more striking than that caused by the injection of an emulsion of normal testicles. The first few rabbits in each series

and all the rabbits in the series from which no virus was recovered served as excellent controls for the work. After 4 to 8 passages, however, in certain series, reactions were noticed in the inoculated eyes, skin and testicles which had not been seen in the earlier animals and which did not occur in the controls. A description of these reactions will be given a little later in the report.

Using the method outlined above a virus has been recovered from 5 of 11 chicken-pox patients. Three of these strains, one of which is now in the 45th generation, have been studied carefully. With the exception of an occasional contamination all the cultures remained free of ordinary bacteria

2. Description of the reactions in rabbits inoculated with the virus.

(a) Eye: Scarified corneas inoculated with the virus showed a reaction which was not present in the controls. This reaction was not so severe as that caused by vaccine virus or the virus of herpes. It appeared in 3 or 4 days and persisted 4 to 5 days. The cornea became rough and opaque, especially along the lines of scarification. There was increased lacrimation, photophobia and injection of the blood vessels around the cornea. The lesions healed rapidly without permanent injury to the eye unless there was a secondary infection. A reaction in the eye occurred less constantly than in the skin. In sections of the inoculated corneas, there was found along the lines of scarification a type of degeneration involving the nucleus and the cytoplasm of the cells similar to the cellular changes found in chicken-pox lesions in the human skin. Occasionally small vesicles were seen in the thick layer of epithelial cells filling the defect in the cornea



produced by the scarification.

(b) Skin: Discrete lesions, following the inoculation of the virus on the scarified skin, were small, superficial, red papules which might easily be overlooked. When the virus was concentrated enough to cause confluent lesions along the scarifications, however, the reaction could hardly be missed. The erythema caused by the scarifications in the control skin disappeared by the 3rd or 4th day at least, while in the inoculated skin the erythema and swelling of the tissues became more evident at this time, lasted 3 to 6 days longer and then rapidly disappeared without leaving any scars.

The intracutaneous inoculation of the virus gave more constant results than smearing it on the scarified skin, especially when the virus was dilute. The virus for intracutaneous inoculation was centrifuged and 0.1 cc. of the supernatant fluid was injected into the skin by means of a tuberculin syringe. The effects from the trauma of inoculation usually disappeared within 48 hours and nothing further was seen when the tests were negative. When the tests were positive, however, an area of the skin often larger than a silver dollar around the point of inoculation became red and slightly edematous. This reaction persisted 4 to 7 days and then disappeared without leaving any scar. Emulsions of normal testicles inoculated into the skin in a similar manner gave no such reactions.

Histological examinations of skin showing the different types of lesions revealed a cellular infiltration of the corium, a swelling and thickening of the epidermis and certain intracellular changes similar to the ones seen in the papular lesions of human chickenpox.

(c) Testicles: The pathological changes in the testicles were disregarded at first because of the amount of material injected. Large quantities were necessary to adapt the virus to animals. Later, however, when the virus was more concentrated, small quantities of the supernatant fluid from thoroughly centrifuged testicular emulsions were injected. Even after these small injections the testicles became swollen and tense in 3 to 4 days. When the testicles were removed for examination or for passages to other animals, they were red, swollen, or edematous with whitish areas scattered over the surface. Upon sectioning them the swollen condition was more evident as the parenchyma bulged out from the restraining capsule. This reaction is similar to that caused by vaccine virus.

(d) General reaction following intratesticular inoculations of the virus: After the virus became adapted to rabbits, we often observed a general reaction in the animals in addition to the local ones in the eye, skin and testicles. The rabbits looked sick, refused to eat, lost weight, occasionally developed a diarrhea and often had a temperature of 104° to 107°. The animals did not always have fever even when good local reactions occurred in the eye, skin and testicles. On the other hand, in some of the animals which had high temperatures, discrete lesions appeared in the control areas of the skin or in the shaved skin of rabbits inoculated only in the testicles. The lesions, red macules and papules of various sizes, appeared 5 to 11 days after the inoculation, remained 3 to 7 days, and then disappeared without scarring. Frequently a zone of erythema less intense than that of the papules surrounded each lesion. These lesions seemed to indicate that some of the virus in the testicles had invaded

the blood stream and had localized in the irritated skin. The demonstration of this invasion of the blood stream was accomplished by removing blood from the hearts of some of the rabbits and injecting it into the testicles of normal rabbits. Four days later an emulsion of these testicles was tested on the skin of normal rabbits for the presence of the virus.

(e) Effects of intranasal, intratracheal, intravenous, and intracerebral inoculations of the virus: No reactions have been observed following the intranasal, intravenous, and intratracheal inoculations of the virus. The animals which were inoculated intracerebrally were sick and had high temperatures for 5 or 6 days. None of them died, however. These experiments are still in progress and a final report can not be given at this time, but it seems that although no signs of infection follow inoculation by the four modes just mentioned these inoculations give rise to an immune state. We feel that in each instance a true infection was set up by the inoculation of the virus even though we were unable to detect evidences of it.

D.- Effect of Certain Physical and Chemical Agents Upon the Virus.

1. Physiological salt solution: Virus preserved on ice in physiological salt solution deteriorates rapidly and after 4 days ceases to give a visible reaction when injected intracutaneously. It is viable under these conditions, however, at least up to 6 days and can be recovered and concentrated by several passages through testicles of rabbits.

2. Glycerine: Virus preserved on ice in 50 per cent glycerine also deteriorates rapidly. But although the virus preserved in this way produces no visible reaction when injected intracutaneously, it

is frequently still viable as we were able to demonstrate by showing that it still immunizes animals and also by recovering it after several passages through animals. We recovered the virus from glycerine 4 times, once each after it had been in the glycerine 7, 17, 29 and 33 days.

3. Heat: We have evidence that the virus is very sensitive to heat; an exposure of 10 min. to a temperature of 55°C. kills the virus, while one of 30 min. to a temperature of 45°C. is not sufficient to destroy it.

4. Filtration: We feel fairly certain that the virus can pass an N filter in the majority of instances. Water suction was used. The filtration was not permitted to go on longer than 30 min. The filters did not allow influenza bacilli to pass through under these conditions. The virus has not been recovered from every filtrate, and, when it was possible to demonstrate the presence of the virus in the filtered material, it was many times more dilute than in the unfiltered.

#### E.- Study of the Immunity Produced by the Virus.

The time of appearance of an immunity to any of the so called filterable viruses probably varies inversely with the amount of virus in the immunizing dose. There are probably many other factors which also influence the time at which the immunity appears, such as the condition of the virus and the point of inoculation. As we had no way of determining the actual amount of virus used in our work we arbitrarily chose to test for the presence of an immunity 2 to 3 weeks after the immunizing dose. A fresh virus well controlled was always used in these tests. In this way we were able to keep at least two factors fairly constant. In these experiments, the virus recovered from vari-

cella patients was always injected intracutaneously in amounts of 0.1 to 0.2 cc., and, the vaccine virus was smeared on the scarified skin. The immunizing strain was inoculated on one side of the animal and the test strain on the other in order to be certain the animal was immune to the former.

Our work on the immunity produced by the virus recovered from varicella patients can be summarized as follows:

1. Three strains of the virus isolated from varicella patients have been studied and each gives complete immunity to the other in rabbits. From this we conclude they are identical.
2. Rabbits immune to the virus from varicella patients are not immune to vaccine virus and vice versa.
3. Rabbits can be immunized by injecting the virus from varicella patients into the skin, into the testicles, into the brain, or into the veins.
4. Old glycerinated viruses which are still viable will immunize animals.
5. Virus killed by heat does not immunize animals.
6. Normal rabbits become immune within 2 weeks after they received small quantities, 2 to 4 cc. of fresh blood from the hearts of rabbits which had been inoculated intratesticularly 4 days previously with the virus from varicella patients. This was considered active immunization.
7. We were unable to demonstrate any passive immunity to the virus in rabbits which 24 hours previously had received intravenously 5 to 10 cc. of serum from rabbits immune to the virus, or had received 5 to 10 cc. of serum from convalescent chicken-pox patients.

8. Serum from immune rabbits neutralizes the virus when it is mixed with the virus and left at 37°C. for 24 hours in the presence of complement. Normal rabbit serum and normal human serum do not neutralize the virus. Serum collected from convalescent chicken-pox patients may have a little neutralizing power, but, because of technical difficulties owing to the numerous titrations and controls which we think necessary, we feel a little hesitancy in making any definite statement about these results.

We believe that we are working with a virus. Furthermore, we are as certain as it is possible for us to be that the virus was recovered from chicken-pox patients under the conditions outlined. It is not the virus of vaccinia or smallpox. While the virus produces lesions in rabbits very much like those of human chicken-pox, we have not shown experimentally that the virus is the etiological agent of chicken-pox. As soon as we can get some fresh cases of chicken-pox we will attempt the active immunization of a number of rabbits by injecting into them the blood of patients early in the disease. If after 2 to 3 weeks these rabbits are immune to our virus we will be warranted in assuming that we have been working with the etiological agent of varicella and, furthermore, we will be warranted in using the virus as a prophylactic measure against chicken-pox in children.

Studies on Physiology and Pathology of the Circulation.

Dr. Cohn, Dr. Murray and Dr. Stewart.

Report by Dr. Cohn.

In this laboratory we have, during the past year, carried on work in two directions, first with tissue cultures made of the hearts of chicken embryos, with the view to studying the behavior of heart

muscle cells or fibers; and second, with dogs, with the view to studying the reaction of the muscle tissue of the heart in them to certain injuries.

The work on the hearts of chicken embryos was carried on with Dr. Murray and with the assistance of Miss Rosenthal. The work was undertaken because it seemed essential from the point of view of the study of disease, to turn to an examination of the function of contraction of heart muscle. In the past twenty years, this study, while not neglected, took a place second in interest to the analysis of other functions of muscle, namely, those which involve the irregularities to which the heart is subject. This study was rewarded with a large increase of success, but failed, I think, to throw light on the nature of the processes which are involved when the heart actually begins to fail. There are several directions from which investigation of this problem may be approached. For the time being we have elected to study it from the point of view of the ageing of such tissue. We have chosen the hearts of chicken embryos because this preparation can be very accurately controlled experimentally.

We found before long that it was necessary to be particular in the eggs we used. With the cooperation of Mr. J. G. Webb, it became possible for us to obtain eggs from a single strain of hens of known age; we know when the eggs were laid and they are sent to us daily under fairly uniform conditions. This makes it possible to be almost certain of the age of the embryos with which we experiment. In order to have a measure with which to compare other functions we studied embryos of various ages to learn whether the tissues at succeeding ages showed differences in their ability to grow. After making a number of attempts, we finally adopted a method by which we can plant

a number of tissue fragments ( 5 to 20) under precisely the same conditions on a single large mica cover glass, covered by a Gabritchowski dish. In preparations such as this, we have the fragments taken from the ventricle, of the same size, planted in the same medium of exactly the same amounts of blood plasma and extract made from the juice of embryos. For a single experiment we now proceed in the following manner. Just before the cultures are made, a chicken is bled and this plasma is used for all the cultures which are to be made. The culture medium, composed of plasma and extract, or Ringers solution, or plasma and Ringers solution and extract, is made with calibrated pipettes. Eggs are then taken containing embryos ranging from 4 to 18 days; a number of fragments of tissue are taken from each embryo and planted on a separate cover glass. All the cultures are under these circumstances exposed to the same medium, the same temperature and the same atmosphere. At stated periods, at 24, 48 and 96 hours, these cultures are magnified by a projectoscope and their outlines are drawn. The drawings are then measured and the results plotted. With the data so obtained we find that we can construct a curve of the rate of growth, which shows that the younger the embryo from which the tissue is taken, the faster is its rate of growth. At 18 days the rate is strikingly slower than at 4 days (Figure 1).

The curve is smooth except at the points representing tissue taken from embryos between the 8th and the 10th days, where there is a flattening of the curve. We have for the moment no explanation to offer of this phenomenon. Over 2000 fragments have now been examined and it is found that a curve of this form is always obtained. The points for cultures of a given age may deviate in different experiments



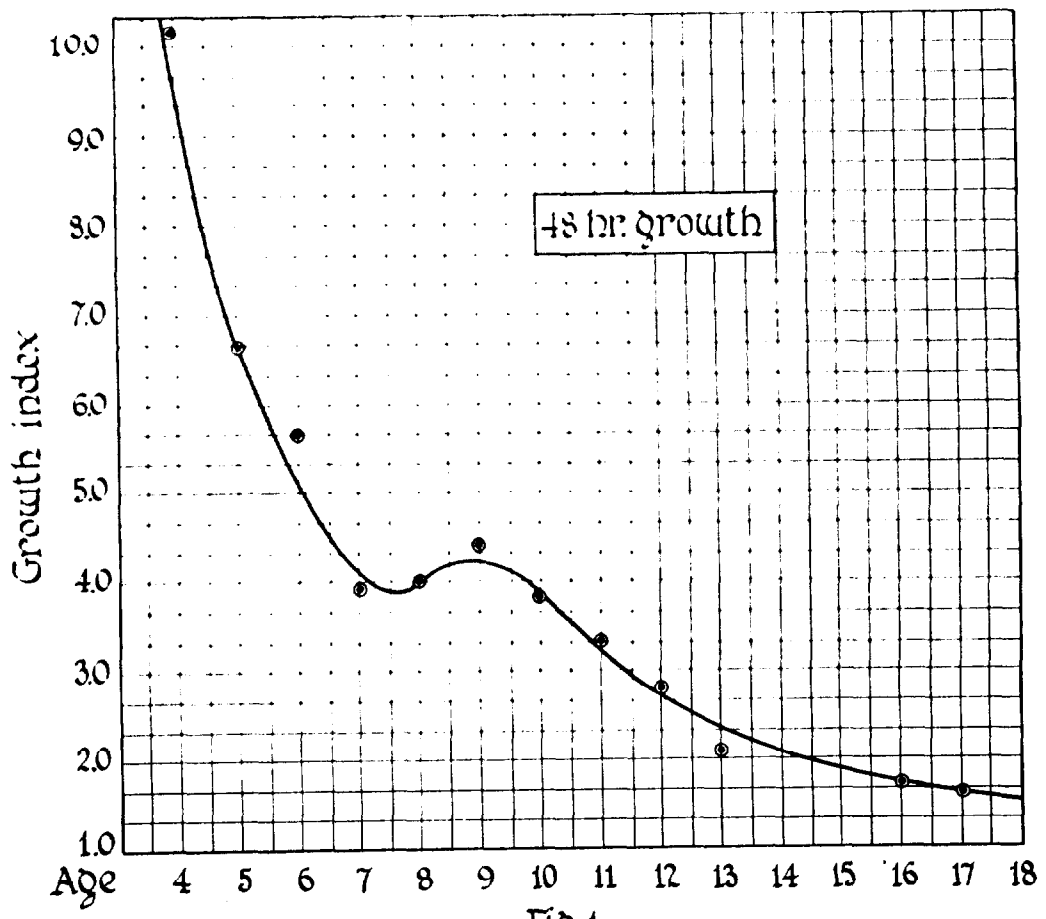


Fig. 1

from those in others, but **the differences** are not material and do not, we believe, disturb the general conclusion to be drawn from the experiments, namely, that the rate of growth of fragments depends on the age of the fragments and that when the rates of the different ages are plotted the points fall on a curve. This curve one might call "The Curve of Potential Growth."

Although the shape of this curve is characteristic, it is characteristic only for the conditions employed; if the conditions are varied the precise, though not the general, shape of the curve is also varied. When for instance, Ringers solution is added to the plasma, it is found that the rate of growth of tissue taken from the older embryos is accelerated. It is of course possible by varying the conditions in a number of ways to vary the curves obtained. But it does not seem important to us to do this, for the exact form of the curve has no significance but only its general shape, and this we believe we have demonstrated. Embryos at succeeding ages have also been weighed, and the percentage increases in weight have been plotted (Fig.2). It will be seen that from the point of view of ageing, the younger the embryos, the greater is the relative increase in weight and in a sense the greater is the rate of ageing; with increase in time this rate slows down so that toward the end of this period, relative increase in weight and in the process of ageing are at a minimum.

We have studied these cultures also in other directions. We have noticed a fact already pointed out by other observers, namely, that after a certain latent period, the fragments in the cultures begin to beat regularly. The fragments from younger embryos begin earlier. Fragments older than 13 days were not observed to beat. The tissue so

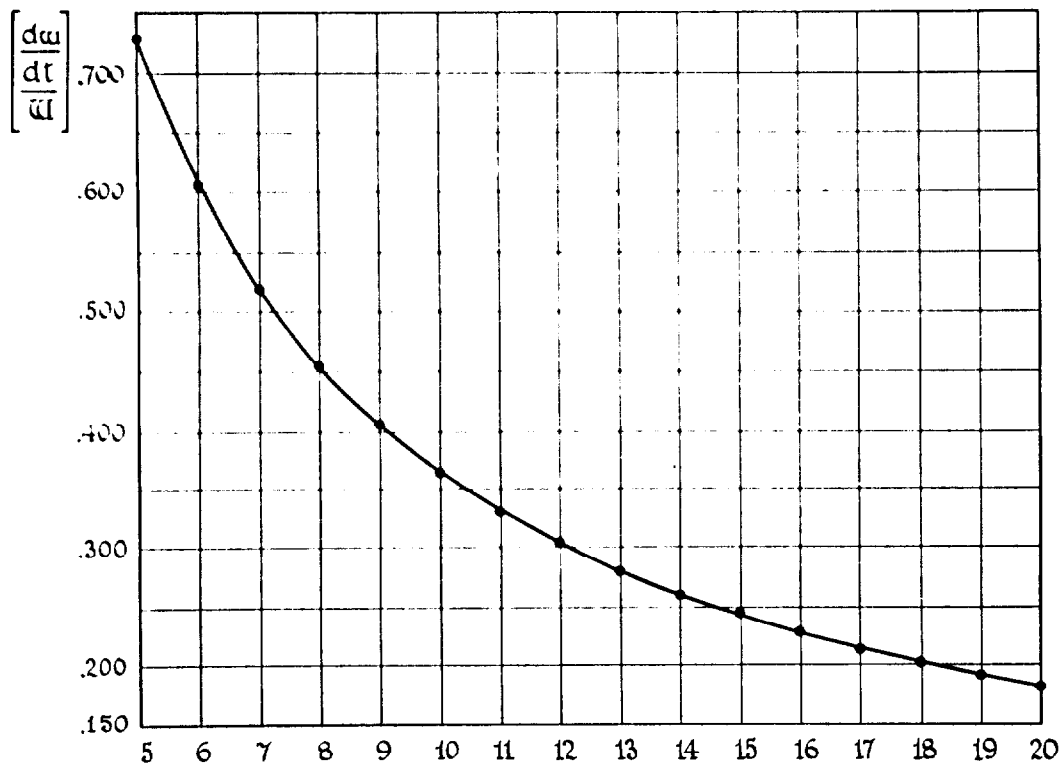


Fig 2

so far studied from this point of view was all ventricular; whether auricular tissue behaves in a similar fashion is not yet known. It is however known that in embryos of above 10 days, the auricles beat faster than the ventricles. Whether there is a difference in the behavior of right and left auricles we do not yet know. Without changing the medium, we have seen individual fragments contract as long as 18 days; usually they continue to do so for 6 to 8 days. We have made preliminary experiments also on the influence of temperature on the rate of contraction, but have postponed further experiments until we can utilize the constant temperature room now building. The attempt to manage such experiments except under properly controlled conditions was too unsatisfactory. Here it will also be easier for us to obtain data on the rate of cardiac contraction of the heart in the intact embryos.

We have in progress experiments with chicken embryos in other directions, reports of which we prefer to postpone for the present; these experiments include studies looking to the construction of curves of dry and wet weights of embryos with advancing years, yielding data on the water content of the embryos; measurements of the transfer during the course of embryonic life of substances from the egg to the body of the embryo; and measurements of the rate of oxygen and carbon dioxide exchange. We might point out that in our plan the examination of these various functions is in each case conceived from the point of view of learning the changes which occur with increase of age. The data should therefore afford a basis for describing the changes in physiology which occur with the process of ageing.

With Dr. Stewart investigation has been carried on to learn what the effects on the circulation are of injury to the heart. Dogs have been used in these experiments. The first step has consisted in devising methods. Although the production of valvular injuries is not new, we are now in a position to produce precisely the lesion we desire. We accomplish this by inserting within the heart, a tubular instrument armed at one end with a lens and a knife. By looking through the tube it is possible to place the incision where we desire. We were about to proceed with making these injuries by an older method, when the description of a more suitable instrument by Graham and Allen was published. Their instrument was not obtainable at the time so that we were obliged to construct one of our own. This we have been able to do and have carried out operations successfully with it. The operation through the open pleural spaces is difficult and infection of the pleura is not unlikely. But we have so far operated on 8 dogs. Three died during the course of operation. Of the remaining 5, 3 are alive after 7, 5 and 4 months.

Although it is possible to produce valvular injuries, an injured valve in dogs does not suffice to bring on a condition of heart failure, as the experiments of other observers have shown. The animals after operation either die of acute heart failure or live without any sign of defective circulation. In addition to the valvular defect it is, therefore, necessary in some way to interfere with the integrity of the heart.

In the first experiments in this direction which we have carried on, we employed diphtheria toxin. This toxin, we have reason to believe on the basis of clinical and of pathological experience, affects the heart. We have accordingly injected varying amounts of

toxin in different groups of dogs; the amounts varying between .001 cc. and .00168 cc. The dogs which received the larger doses died; with one exception, the dogs receiving the smaller doses survived.

We devised a method for measuring the size of the hearts by X-rays, comparable to that previously employed in human beings. With this technique it is possible to ascertain changes in cardiac size during the life of the dogs.

Aside from developing jaundice as the result of the injections, and of losing weight, we soon learned that the hearts of these dogs decreased materially in size. At first we thought that the loss in size went parallel with the loss in weight, but in a series of dogs which were first starved, then brought back to the initial weight and subsequently injected, there was no decrease in the heart's size as the result of starvation, comparable to that which occurred as the result of injection. It was clear therefore that the diminution in size had to do with the intoxication. To account for this action of the toxin, a number of possibilities presented themselves. The decrease might be due (1) to destruction of the substance of the heart; (2) to destruction of the blood; (3) to decrease in the blood volume. The attempt to relate decrease in the hearts' size with decrease in the volume of the blood or with destruction of the blood failed. A change in the size and regularity of the red cells took place at least on one occasion, but we do not know the significance of this change to the condition we desired to explain. That diminution of the blood volume might be expected to influence the size of the heart as seen in the X-ray is shown by the results we obtained by bleeding a dog, for after withdrawing 500 cc. from an animal the size of the heart's shadow decreased 25%.

Synchronously the count of the red cells decreased, from which we infer that the organism attempted promptly to make up the loss in blood volume by diluting the plasma. The blood taken from the first animal was transfused into a second dog, proper precautions as to agglutination having been taken. By injecting this amount one would expect the heart of the second dog to increase in size. An increase did in fact take place, to the extent of 9%; but this half of the experiment has not the validity of the first half for the body has facilities for storing blood, whereas it has none for making good a rapid loss such as we imposed. Although small changes in the histology of the heart muscle were found, the extent of this is not sufficient to account for the decrease in size we detected, neither did the weight of the whole heart decrease beyond the extent anticipated by decrease in the body weight.

All these examinations leave us without an adequate explanation of what causes the decrease in heart size. Two other possibilities present themselves which we have not explored; (1) the possibility that the diphtheria toxin dilates the capillaries and that the blood disappears into them with consequent decrease in the size of the heart; and (2) the possibility that water passes out of the muscle tissue causing the appearance we found.

Whatever the nature of the change, we have, it appears an agent which if added to the valve injury we can produce, is capable of decreasing the integrity of the muscle to the extent desirable in conducting these experiments. We ought accordingly now to be able to prepare dogs so that they may present the aspects of failing circulations and make it possible to study in detail the alterations in the circulation which occur under these circumstances.

Studies Carried on in the Chemical Laboratory on Nephritis and Cyanosis and Physical Chemistry of the Blood.

Dr. Van Slyke, Dr. Lundsgaard, Dr. Hastings, Dr. Salvesen, Dr. Linder, Dr. Heidelberger, Dr. Neill and Miss Hiller.

Report by Dr. Van Slyke.

Cyanosis.

A review and theoretical consideration of the causes of cyanosis has been prepared for publication by Lundsgaard and Van Slyke. Lundsgaard had previously demonstrated that the factor responsible for the production of cyanosis is the concentration of reduced hemoglobin in the capillary blood, and our study resolved itself into an investigation of the quantitative effects of the factors contributory thereto. The contributory factors are (1)  $\int$ , the degree of oxygen unsaturation of the arterial blood coming from aerated lung areas; (2)  $\frac{d}{l}$ , the proportion of blood passing from the right heart to the left through unaerated channels; (3)  $\frac{D}{V}$ , the oxygen consumption in the capillaries; (4)  $T$ , the total hemoglobin content. In effectiveness in causing the presence of reduced hemoglobin in the capillary blood, these four factors rank in the order named. For example an increase of 50 per cent of the distance between normal and maximum in each factor increases the capillary reduced hemoglobin content from the normal 2.6, expressed in grams per 100 cc. of blood, to 9.3, 6.4, 4.5, and 2.8, respectively, 4 to 6 being the range at which cyanosis usually becomes visible. The combined effect of the factors was found to be expressible by the equation

$$C = \int T + \frac{dD}{1-d}$$



There are various other factors which modify the coloration. Such are local skin vascularity, pigmentation, thickness of epidermis.

The main clinical conditions in which cyanosis is a symptom have been considered in connection with the causative and modifying factors present, and attempts have been made to estimate the functional and anatomical significance of the cyanosis in certain of these conditions.

Nephritis.

In the nephritis work Linder and Lundsgaard have continued their studies of blood protein and blood volume changes. They find in glomerular nephritis and nephrosis (terms used in the sense of Volhard and Fahr) that the total protein content of the plasma is usually reduced below the normal; it varies from 3.5 to 5.5 grams per 100 cc. compared with the normal 6 to 7. In all the cases of these two types studied a fall in the albumin ratio below the normal occurred.  
globulin

Since albumin is the protein chiefly excreted in the urine, the readiest explanation for its relative (and usually absolute) deficit in the plasma is direct loss by excretion. Whether this is the entire explanation is uncertain, however. The excretion of albumin and globulin in the urine of these patients is being studied with the above point of view.

In cases of nephrosclerosis and functional albuminuria the plasma proteins were normal.

The cause of the low concentration of proteins in the plasma content observed in many nephritics has been an object of speculation since the time of Bright. There were two possibilities: either there was a loss of protein from the body, or the blood was diluted with

retained water (hydremic plethora). In order to obtain data that might lead to a decision, blood volume determinations by Keith, Rowntree, and Geraghty's "Vital Red" method, repeated at intervals over varying periods of time have been performed on patients with low plasma proteins. In no instances, even when there was great edema, has the blood volume been found abnormally high. We have thus far had no cases in which a plethoric hydremia existed. The low protein content appears due entirely to a diminution in the total amount of plasma proteins in the body. The latter, calculated from the protein concentration and the plasma volume, have been found to be about 3.5 grams per kilo body weight in normal subjects. In the nephritics with low plasma protein concentration the figure varied between 1.5 and 3.0 grams.

In some cases a rise towards normal of the total plasma proteins was observed to accompany clinical improvement. The increase in plasma proteins did not occur until after edema had disappeared. It does not appear probable that the low plasma protein content is a factor in the production of edema.

#### Blood Gases.

With Dr. Neill the details have been perfected for utilizing the "constant volume" blood gas apparatus for all blood gas analyses. The gases are extracted in the same manner used with the former "constant pressure" apparatus. The measurement of the gas, however, is based on the principle of reducing it to an arbitrarily chosen, convenient, definite volume (5 cc., 2 cc., or 0.5 cc.), and measuring on a mercury manometer the pressure which the gases exert when compressed to that volume. The amount of gas present is proportional to the pressure. In the former apparatus the pressure of meas-

urement was constant (at atmospheric), the mass of gas being measured by the volume. In the present apparatus the volume is constant, the mass of gas being measured by the pressure. A great gain in accuracy is obtained, because we can now choose conditions of measurement such that the error in measuring the volume of gas shall be no greater than the error in measuring the pressure, e.g. with the volume of gas at 5 cc., as in our most accurate  $\text{CO}_2$  determinations, and the pressure to be measured at 200 to 300 mm. of mercury, both volume and pressure can be measured to 1 part per 1000, and the sum of errors in both measurements is only 2 parts per 1000. The manipulation of the apparatus is so simple that this degree of accuracy is, in fact, attained in our routine determinations. Refinement to this point was forced upon us in order to obtain data from which we could determine the effect of oxygenation and reduction on the base-binding power of hemoglobin.

#### Physical Chemistry of Hemoglobin.

As reported in October, we found that at pH 7.4 the sodium salt of oxyhemoglobin binds 2.15, reduced hemoglobin 1.47 equivalents of Na per molecule of hemoglobin, the difference being 0.68 equivalents of alkali; i.e. if reduced hemoglobin is oxygenated, its acidity is augmented so that it binds at the same pH 0.68 more equivalents of Na per molecule. However, we found that in blood the change from complete reduction to oxygenation increased the alkali bound by proteins only by 0.56, instead of 0.68 equivalents per molecule of hemoglobin. This difference we were unable to explain at the time.

We believe, however, that it is now completely explained. Hastings, Neill, and Harington have found that in hemolyzed blood

the same effect of oxygenation is obtained as in the solutions of pure sodium hemoglobinate. The formerly observed difference between the solution and the blood is therefore attributable to the confinement of the hemoglobin in the cells of the blood.

Physical Chemistry of the Entire Blood.

The work done in Peking by Van Slyke, Wu, and McLean, and here reported was a development of the studies of the physical chemistry of the blood on which this laboratory is engaged. It has been known that at normal pH the chloride and bicarbonate in the cells are only about half as concentrated as in the serum although both Cl and  $\text{HCO}_3$  pass readily through the cell walls. On the other hand the hydrogen ion concentration is greater in the cells than in the serum although the cell membranes appear permeable to  $\text{H}^+$  ions. By some mechanism it was known that the Cl and  $\text{HCO}_3$  distribution is so regulated that it enables the indiffusible cell buffers (hemoglobin) to act through the cell wall and assist in maintaining the neutrality of the serum with nearly the same degree of efficiency that the hemoglobin would exert were it directly dissolved in the serum. We had no explanations, other than vitalistic ones, for these phenomena, nor for the fact that rise of  $\text{CO}_2$  tension causes the cells to take water from the serum. The discovery of the magnitude of the amounts of alkali with which hemoglobin combines, and the great effect of changing pH on the alkali combined, indicated the manner in which a common explanation could be found for the above phenomena, on the basis of the known laws of solutions. The problem was solved by combining the three physicochemical laws which govern the distribution of electrolytes between solutions at osmotic equilibrium on two sides of a membrane, which is permeable for only part of the ions present. These

laws may be expressed, for the conditions found in the blood as follows.

1. For electrical neutrality cations and anions must be equal at reactions as near the neutral point as the blood  $H^+$  and  $OH'$  ions are negligible, and the base is combined partly with monovalent anions ( $Cl'$  and  $HCO_3'$ ), partly with protein. Representing the concentration of total cell base as  $[B^+]_c$ , that of the monovalent anions as  $[A^-]_c$ , that of the negatively ionized protein, or protein combined with alkali, as  $[P^-]_c$ , and similar values in the serum as  $[B^+]_s$ ,  $[A^-]_s$ , and  $[P^-]_s$ , we have, assuming complete electrolyte dissociation,

$$[B^+]_c = [A^-]_c + [P^-]_c$$

$$[B^+]_s = [A^-]_s + [P^-]_s$$

II. According to Donnan's law, when electrolyte solutions are separated by a membrane permeable for only part of the ions, the monovalent permeable ions so distribute themselves that the ratio of the concentration of any anion in the cell to the concentration of the same anion in the serum is the same as that of any other anion, and the reciprocal of that of any cation. For the permeable ions in blood, therefore, we have the following distribution:

$$\frac{[H^+]_s}{[H^+]_c} = \frac{[Cl^-]_c}{[Cl^-]_s} = \frac{[HCO_3^-]_c}{[HCO_3^-]_s} = \frac{[A^-]_c}{[A^-]_s} = r$$

the letter "r" being used to indicate the common ratio.

III. For osmotic equality between cell contents and serum the concentration of osmotically active ions and molecules in each must

approximate equality. We found that the unit of osmotic concentration is not the ratio dissolved substance / volume of solution but dissolved substance / water,

so that  $[Cl]_c$  represents the equivalents of chloride per kilo of water in the cells, not the chloride per liter of cells. The total concentration of the osmotically active substances in the serum may be represented as  $2[Ba]_s + [BP]_s$ , since the salts represented by Ba, are dissociated into two osmotically active ions  $D^+$  and  $A^-$ , while the protein salt  $[BP]$  dissociates into ions of which only one  $B^+$ , has important osmotic activity,  $[P^-]$  having so little that it may be neglected. In the case of the cell contents we must add to the osmotic activity of the electrolytes that of the hemoglobin, which is about 1/10 of the total. Therefore, if the osmotic concentration in cells and serum are even we have

$$2[Ba]_s + [BP]_s = 2[Ba]_c + [BP]_c + [Hb]_c$$

The accuracy of this equation was demonstrated by analyses of cells and serum.

By combining the above equations we obtain finally one which expresses the relationship between the electrolyte distribution and the alkali bound by the blood proteins, viz.

$$r = \frac{[H^+]_s}{[H^+]_c} = \frac{[Cl]_c}{[Cl]_s} = \frac{[HCO_3^-]_c}{[HCO_3^-]_s} = 1 - \frac{[BP]_c + [Hb]_c - [BP]_s}{2([Ba]_s - [BP]_s)}$$

This equation was tested quantitatively by analysis of the serum and cells of blood after it had been subjected to varying  $CO_2$  tensions. It was found to agree nearly within the limit of experimental error in  $H^+$ ,  $Cl^-$ , and  $HCO_3^-$  ratios.

The water distribution was calculated as follows: the equation of osmotically active substances may be written as

$$2 \{B\}_s - \{BP\}_s = 2\{B\}_c - \{BP\}_c + \{Hb\}_c$$

or if we use  $(B)_s$  to indicate the serum base,  $(B)_c$  the cell base, etc., per kilo of blood, we may write it as

$$\frac{2 (B)_s - (BP)_s}{(H_2O)_s} = \frac{2 (B)_c - (BP)_c + (Hb)_c}{(H_2O)_c} \text{ or}$$

$$\frac{(H_2O)_s}{(H_2O)_c} = \frac{2 (B)_s - (BP)_s}{2 (B)_c - (BP)_c + (Hb)_c}$$

The distribution of water between serum and cells was found within the limits of analytical error to be related to the amount of base bound by the cell proteins in the manner indicated by the last of the above equations.

#### Electrolyte Distribution and Osmotic Pressure between Blood Serum and Edema Fluid.

The laws above discussed governing the distribution of salts and water between serum and cells in the blood appear sufficient to explain also the distribution between the serum and edema fluid. The equation expressing the theoretical relationships differs from that developed above, because not only the anions, but also the alkali cations appear freely diffusible through the membranes separating blood from edema fluid. It is similar, however, in predicting a smaller Cl and  $HCO_3$  concentration and a greater Na concentration in the serum than in the fluid. Analyses of serum and fluid recently published by Atchley, Loeb, and Palmer yielded results which agreed with the distribution

values predicted, as closely as the latter could be calculated from the data. More complete analyses of serum and fluid from edematous patients have been made by Hastings and Salvesen, who find that the agreement between the predicted and observed Cl and  $\text{HCO}_3$  ratios is quantitative, and that between the Na ratios is approximate.

The osmotic pressure theoretically predicted cannot be equal on both sides of a membrane when, as in this case, all the anions and cations except protein fail to diffuse through. The pressure must be towards the solution with the greater protein content, viz. the serum. Dr. Hastings has prepared collodion membranes simulating in permeability the body membranes that separate serum and edema fluid. With these membranes and a micro-osmometer he has devised, he has determined that the osmotic pressure in the serum exceeds that in the edema fluid by 15 to 20 mm. of mercury.

The osmotic pressure is, therefore, in the direction that would cause fluid to pass from the tissue spaces into the serum. It consequently appears that forces other than osmotic pressure are involved in the passage of fluid in the direction from the blood to the serous cavities and intercellular spaces.

Salvesen is studying the mineral and protein composition of nephritic serum. He finds that among the mineral constituents, calcium is peculiar in that it parallels the protein content. It also exists in higher concentration than it can attain in protein-free solutions containing carbonate and phosphate concentrations equal to those in serum. At times in nephritis the calcium falls as low as in parathyroidectomized dogs with tetany. Yet the nephritic may not have tetany. A preliminary explanation of the phenomena is that a large part of the serum calcium



is combined in non-ionized form with protein. It is this part that varies parallel to the protein content. The ionized calcium, on the other hand, may be the part that is lost by parathyroidectomized animals, lack of it causing tetany.

To test the probability that slightly ionized calcium protein compounds exist in the serum, Hastings, Murray, and Miss Hiller are engaged in preparing calcium salts of the separate serum proteins. The degree of dissociation of these salts will be measured by means of the solubility product method, and if possible by the electrometric method also.

The method of Heidelberger for preparing crystalline horse hemoglobin has been applied successfully by him to preparing hemoglobin from bloods of the guinea pig, rat, sheep, and donkey.

#### Study of Rheumatic Fever.

Dr. Swift, Dr. Miller and Dr. Boots.

Report by Dr. Swift.

The clinical study of rheumatic fever has been continued in the directions previously outlined. Because of the close relationship between this infection and chronic heart disease it is necessary to follow all of our patients for long periods after recovery from the acute attack. The real life history of the malady can, therefore, be written only after many years, for only by repeated observation of the same individuals can the influence of certain abnormalities in the first stages of the infection be correlated with later pathological processes. Thus, for example, previous clinical studies indicated that from 30 per cent to 50 per cent of patients with rheumatic fever develop signs of endocarditis or chronic cardiac valvular disease. The studies by Dr. Cohn and myself, mentioned in previous reports but completed with-

in the past year, show that at least 90 per cent of our patients had abnormal electrocardiograms while under observation in the wards. These results indicate either that the heart muscle, the impulse conduction system and branches of the coronary artery are affected during the acute disease more often than is the lining membrane of the heart, or that our methods for detecting myocardial disturbance are more accurate than those for detecting endocardial injury. These instrumental signs of cardiac involvement are often only temporary; lasting from one day to a week, a period of time similar in duration to that often seen in a single inflamed joint of a patient with rheumatic arthritis. This suggests that some of the cardiac lesions, at least, are similar in nature to those present in the joint tissues. The relation of these evidences of transitory lesions to the more deep-seated injuries found in chronic heart disease is the subject of investigation at the present time.

The histopathology of rheumatic fever is almost entirely founded upon study of material obtained postmortem, and hence from subjects who were victims of the chronic forms of the disease, especially those who had succumbed to heart failure. The heart, therefore, has been the principal organ studied. In recent years subcutaneous fibroid nodules removed both postmortem and during life have been studied microscopically. Two pathologists have reported upon the investigation of joint tissues obtained postmortem. Because of the desirability of knowing the nature of the earlier lesions we have, during the past year, excised small portions of tissue from acutely inflamed joints of patients in the early days of an attack of rheumatic fever and submitted them to histological and cultural examination. So far, the cultures have been sterile. The microscopic exa-

mination reveals small foci of inflammation in all of the joint tissues, but most marked in the synovial membranes. A very striking feature is the involvement of small blood vessels consisting at times of occlusion of the vessels with proliferated lining cells, and at other times of marked accumulation of endothelial cells together with polymorphonuclear leukocytes in the perivascular spaces. This implication of the smaller blood vessels is a feature of most of the lesions of rheumatic fever, - whether in the brain, heart, subcutaneous tissues, or joints, as a part of chorea, myocarditis, rheumatic nodules, or arthritis, respectively. It is probable that other structures such as the muscles, and possibly the kidneys, are similarly affected by the virus of the disease.

Dr. Miller and I are attempting to reproduce some of the lesions of rheumatic fever in rabbits, and are using the type of reaction above described as the basis for comparison. For inoculum we have employed rheumatic heart valves, subcutaneous fibroid nodules, and joint fluid. We have introduced the inoculum into the veins, peritoneal cavity, anterior chamber of the eyes, and into the testicles. By intratesticular inoculation a mild interstitial orchitis has been produced in certain series of rabbits and carried along through several generations. The optimum conditions for the production of these lesions are now under investigation and several extensive series of controls are under observation. The optimum time for transfer seems to be about a month. Shorter periods have resulted in a failure to carry on the peculiar lesions. The report of the results obtained so far can only be considered preliminary in nature; but the findings are sufficiently encouraging to continue work along this line.

Drs. Boots and Miller have completed a therapeutic study of neocinchophen (ethyl ester of paramethyl phenylcinchoninic acid) and novatophan (ethyl ester of phenylcinchoninic acid). These two drugs are closely related; the former, also appearing under the trade name of tolysin, is being extensively advertised as a noninjurious substitute for the salicylates in the treatment of rheumatism. Some observers in toxicological studies with laboratory animals have reported that only subtoxic doses are absorbed from the gastro-intestinal tract. Our studies with patients, on the other hand, indicate that doses of the drug sufficient to control completely the evidence of arthritis and of general infection are not infrequently accompanied by slight irritation of the kidney, and occasionally by severe albuminuria and numerous casts. Other mild toxic symptoms are also seen. Moreover, when the drug is discontinued a certain number of patients have had relapses in a manner similar to that seen at times following the withdrawal of salicylates. Occasionally a patient is encountered who does not respond well to neocinchophen but who shows marked effect from the salicylates; likewise, neocinchophen can sometimes be safely and efficiently used in patients with a marked idiosyncrasy to the salicylates. There is no doubt that these newer drugs are valuable additions to our therapeutic armamentarium; but, in so far as we can determine, their action is similar to that of the older salicylates in that they seem to have a favorable action upon certain of the distressing manifestations of the disease rather than a definite curative effect.

Dr. Boots and I have completed a study of joint sensitization with nonhemolytic streptococci. It has been previously claimed by Henry and by Faber that the joints of rabbits may be rendered more

sensitive to invasion of these cocci from the blood stream if the joints were previously injected with small amounts of killed homologous cocci or extracts of these micro-organisms. Upon the basis of these experiments they explained the pathogenesis of rheumatic arthritis. The recent work of Besredka and of Gay, on the other hand, would indicate that these tissues might be immunized by this local treatment. Our work has failed to demonstrate either a sensitization or increased resistance to infection in joints injected with either vaccine or bacterial extract. Joints so treated were about as liable to involvement as the other joints of the same animals after inoculating them intravenously with living streptococci. We, therefore, do not think any experiments up to the present time prove that the arthritis of rheumatic fever is a phenomenon of hypersensitization.

Mrs. Lancefield and I have been trying to demonstrate immune bodies against nonhemolytic streptococci in the blood of rheumatic fever patients. As a starting point we used the results of Kinsella and myself, viz: - (1) That certain nonhemolytic streptococci possess extensively reacting substances so that using extracts of them as reagents with complement binding tests the presence of immune bodies in many rabbits inoculated with different strains of streptococci could be demonstrated. We might designate such strains as "master strains". (2) In the blood of patients suffering from streptococcus viridans endocarditis it was possible to demonstrate complement binding antibodies when extracts of the homologous strain obtained in blood cultures were used as antigens.

We had never tested for cross fixation with the serum of these patients and the "master strains" of streptococci; so that in

the past year we obtained serum and blood cultures from patients in Mt. Sinai and St. Luke's hospital for this purpose. In spite of continued efforts to find them, only a few cases were available for these tests. Much time was consumed in preparing antigens that would react satisfactorily with the serum of patients. The results with the few cases available indicate that, with previously devised methods, complement fixing antibodies could be demonstrated only against the strain of streptococci producing the endocarditis in a given case, but not against the "master strains."

We then prepared extracts of nonhemolytic streptococci by the method recently devised by Avery and Heidelberger for the demonstration of the nonspecific "P" substance of pneumococci. This method seems to give a more extensively reacting antigen, but we have not had enough material from patients with streptococcus endocarditis to test it thoroughly.

We have also tested the serum of a number of rheumatic fever patients with our "master strains", but up to the present time have been unable to demonstrate any definite antibodies against streptococci. This failure may be due to (1) insufficiently sensitive reagents; (2) absolute specificity of reaction between antibody and homologous antigen; or (3) to absence of antibody against streptococci due to the possibility that these organisms are not the etiologic agents in this disease. Until our methods are more extensively tested we shall be unable to decide which of these possibilities must be eliminated.

The Pathological Laboratory.

Dr. Branch.

During the past year there have been 10 cases autopsied. 5 of these cases came from the respiratory service, 3 from the rheumatic

3 from the cardiac, and one from the nephritic. They include:

3 cases of chronic valvular disease of rheumatic origin, Aschoff Bodies having been found in all cases;

1 of coronary artery thrombosis of non-specific origin;

2 septicemias, one due to a meningococcus and the other to a staphylococcus. In both of these there was a purulent arthritis found.

1 case of subacute bacterial endocarditis, the etiological agent being a hemolytic hemophilic bacillus.

2 cases of lobar pneumonia - pneumococcus type I and III being isolated, respectively. The case associated with the pneumococcus type I was a serum treated individual in which an organizing pneumonia was present and an unusual form of pneumococcus endocarditis of the mitral valve.

1 case of pyelonephritis (staphylococcus) engrafted on a nephrosclerotic kidney. This latter conforms to the type of diffuse sclerosis (arterio-capillary fibrosis (Gull and Sutton) or diffuse hyperplastic sclerosis (Evans) ) as vascular changes in spleen and heart are also present as well as those in the kidney.

Two of the above cases have been written up for publication: one with Dr. Miller - "Subacute Bacterial Endocarditis Due to a Haemolytic Haemophilic Bacillus (Haemolytic Influenza Bacillus?)", and the other with Dr. Stewart - "Rheumatic Pancarditis, with Calcification of Myocardium and Mural Endocardium."

Of 2 surgical specimens one, a gland from a case of infective mononucleosis, was most interesting.

Dr. Branch has also been engaged in studying the pulmonary

lesions produced in mice by exposing the mice to atmospheres containing pneumococci in suspension. This study has been discussed previously.

RUFUS COLE.