REPORT OF THE DIRECTOR OF THE HOSPITAL

April 21, 1923

REPORT OF THE WORK OF THE HOSPITAL.

April 1923.

During the period covered by this report patients suffering from the following named diseases have been admitted to the hospital and various problems relating to these diseases have been studied: Heart Disease, Nephritis, Acute Rheumatic Fever, Chicken-pox, Acute Respiratory Infections.

Heart Disease. (Report of Dr. Cohn.)

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

With Dr. Murray, a study of muscle contraction has been begun. It is the general object of this research to arrive at some description of the behavior of muscle relative to certain changes in the life history of this tissue and to the nature of its environment under various circumstances. As criteria of behavior we count on its reaction in terms of growth, of rate and of contractile power. The method of approaching this problem has given us much concern for, so far as human physiology in especial is concerned, muscle may be regarded from two aspects; first its relation to the process of ageing and senescence; and second its relation to the processes of disease. It is increased information on these matters in particular at which we wish to arrive. In the second case there are included at least two phases; that involved in hypertrophy

of the fibre and that involved in increase of its length.

It seemed desirable to study first the relation to ageing. For this purpose it is advantageous to confine the investigation especially at first to a single species, such as the fowl, since material is readily available throughout embryonic as well as in post-embryonic life. We were glad to make this choice not only on this account but because of the great experience with it which Dr. Carrel has accumulated and which he has placed unreservedly at our disposal.

We have, in the first place, devoted considerable time to becoming familiar with our material. In choosing the direction of our work we thought it important first to learn the influence of age on the rate of growth of tissue. In a sense this is not unlike that aspect of the subject which Dr. Carrel has developed in studying the inhibiting influence on growth of plasma taken from fowl of increasing ages. In our work, we are on the other hand, studying the rate of growth of which the tissue is capable at various life periods. With this, we hope to be able to compare certain other properties of heart muscle, more directly concerned with contraction.

In our early experiments we planted the tissue in hanging drops composed of chicken plasma and of extract made of chicken embryos, each fragment being placed on mica covers closed in by separate hollow ground glass cells. Later, so that we might assume a like influence of a number of variables, we planted a number of fragments (5 to 20) in the same medium on large mica covers, supported on large dishes (Gabritschewski). This gave us the opportunity of averaging the performance of a number of fragments and of studying the deviation of each from this average. We found that, although this method lent itself to the construction of a curve of growth, the rate of growth decreasing with the age of the embryo, the de-

viations of individual experiments from the average were greater than we supposed could be accounted for by variations in technique from one to another experiment.

The basis of the differences appears to lie in the nature of the eggs with which we were supplied. We have accordingly soughtand obtained the cordial cooperation of Mr. J. G. Webb, who has supplied us with the eggs we require. With this help we have succeeded in eliminating certain errors. We now know exactly the history and age of the hens, the eggs of which we examine; we know the time at which the eggs are laid; we receive the eggs daily, within 24 hours of the time they are laid. These are the eggs we are now utilizing but they have not been available long enough to permit us to report the results.

Directly connected with this study, we are estimating the water content of the tissue through embryonic life. The embryos are weighed before and after drying. In measurements already made, it was found, just as in the case of tissue growth, that a curve could be constructed showing a relation between age and growth of the embryo as a whole. But here again it was thought desirable to carry on the work with more accurately known material. For this reason the work was postponed until now, when the eggs already referred to supplied by the Webb Farm have become available.

We hope to relate these studies to the histology of muscle at different ages and also to certain other functions. We have therefore begun to study rhythmic contraction. For this we have preparations of two sorts. In the first it was our object to learn whether under the conditions of tissue culture a preparation could be obtained to serve as a reliable control. Fragments of tissue were taken from the hearts of embryos at various ages, and were planted in a medium consisting of chicken

plasma and embryonic tissue extract. We have so far not experimented with types of media, of irrigation, or of variations in oxgyen tension nor with the results of transplanting the tissue frequently to new media. We do not know how long tissue may live when these factors are taken into account. Fragments of tissue have been taken from the two auricles and from the ventricles, especially the left one. In these cultures, certain fragments, usually more than half from each of these localities, contracted; they contract for periods of time ranging from one to fifteen days. It is usual for them to continue to do so for six to eight days; it is unusual for them to do so for a less period. Usually the fragments contract as soon as they are planted; sometimes they do not begin until after a lapse of two to three days. Often the rate slows after a few days, but later rises again. We have noticed that the rate at which auricular tissue contracts is greater than that taken from the ventricles. There seems also to be a difference in the rate of contraction between right and left auricles. We have done a few experiments to observe the effect of alteration of temperature on rate and have seen that in these fragments temperature exerts an influence on rate, an effect frequently observed by others in other preparations. We are, however, not yet able to say whether in our preparations the rate increases with temperature according to the laws of a simple chemical reaction. The differences in behavior of auricles and ventricles, and the possible difference noticed between the two auricles, suggest either that these differences indicate differences in the structure of the muscle taken from various regions of the heart, or that the differences observed depend on the presence of other tissues such as ganglion cells or nerve tissue incorporated in the muscular structure. These are matters for future study. They recall those observations in embryology bearing on the irwandering of ganglion cells into the

heart muscle. As to the effect of the presence of these cells on the function of the heart muscle as apart from the formation of electrocardiograms, little or nothing is known.

In addition we believe it to be important for us to study the rate of the whole heart in the intact embryo while this is still undisturbed in the egg or, at least, disturbed as little as possible. For this we are employing the galvanometric method of registering the heart beats. The method has been used before now, but it has been used to study the form of the electric curve which is obtained. Little attention has been attached to the question of rate. But it is this function of the muscle which to us is important in the attempt to establish those relations to other functions, such as metabolic rate, to which reference has already been made in discussing growth, age and tissue density.

The subjects which I have mentioned cover a wider field then can be investigated immediately. I have described them so as to indicate the point of view from which at the present time we are approaching this problem. Having arrived at these general views and explored the possibilities afforded to us by our material, we intend first to make the measurements which we hope will permit us to construct growth curves; next to learn the natural rate of the heart during embryonic life; and next to ascertain the validity of those preliminary observations, already referred to, on rate exhibited by tissue from various portions of the heart, and the influence of temperature upon it.

With Doctor Stewart experiments are in progress on dogs with the view to producing valvular lesions in them so that information on certain circulatory problems connected with the volume and minute outputs and with the rate of flow, may be gained. Lesions of the valves in dogs that survive have of course been made, notably with such pieces of apparatus as the valvulotome. It was our intention to use this instrument, although the precise

lesions to be obtained with it could not be predicted. Meanwhile, during the autumn (1922), a notice appeared in the Journal of the American Medical Association by Graham and Allen stating that they had made and used an apparatus not unlike a cystoscope with which it is possible, when introduced into the heart through one or the other auricle, to cut the valves and to see precisely what one is cutting. The instrument used by them was not available at the time. Accordingly we set to work to devise one here and have now succeeded in producing one which meets our requirements, giving a large field of vision in the area of operation. So far Doctor Stewart has succeeded in operating successfully on two dogs which have survived. The risks of the operation are great as Graham and Allen reported. We also found this to be true during the time when the intracardioscope was being perfected. Since then, subsequent operations have shown that the dangers appear not to be unduly great. The aseptic technique is, as is well known, difficult of attainment because of the prolonged and wide exposure of the pleural cavity in the approach to the heart. The dogs so far operated on have survived 10 and 7 days. There is reason to believe that we shall succeed in preparing the sort of dog required for our subsequent studies.

It is however not enough merely to produce a valvular defect in order to bring about a state of chronic heart failure as other experimenters, notably Cushing, have pointed out. It is the usual experience, and it was mine here, that the degree of failure after injuring the valve either is extreme resulting promptly in death or that heart failure fails entirely to appear. In view of the fact that this is still likely to be our experience, we mean to resort to the device of subjecting the animals which survive the operations to one of several procedures, notably to work on a treadmill. But meanwhile we have studied the effect of another method. We plan, in addition to producing valve lesions, to cause the muscle of the dogs to degenerate.

In rabbits a variety of agents injure the heart muscle, but there is as yet little experience in this direction in dogs.

We have resorted in the first instance to the use of diphtheria toxin, and have met with results we did not anticipate. A number of control measurements were first obtained such as electrocardiograms, X-ray photographs and the body weight. It was found that if 0.0016 cc. per kilo of body weight of diphtheria toxin was injected intravenously the dogs became ill, failed to eat, lost weight and died. The duration of life after injection was from 3½ to 7½ days. During this time it was noticed that the skin of the dogs became discolored, the urine took on a yellow-brown color due to bile, the red cells underwent changes so that in the smear they presented marked variation in shape and size. Very striking was the reduction in the size of the hearts. This was determined from X-ray plates by measuring the size of the hearts with the planimeter. In view of the fact that after the injections the animals failed to eat, it was thought necessary to exclude the possibility that the reduction in size was due to starvation. With this phenomenon we were familiar from studies made in cases of diabetes mellitus treated by the method of undernourishment. We starved dogs accordingly for the same period of time (4 days) in the course of which reduction in size after injection had been observed, and found that starvation of this duration produced no greater change in the size of the hearts than could be accounted for by the inaccuracy of the method, which Dr. Levy pointed out. The hearts of the same dogs subsequently showed reduction in size when toxin was injected. Other dogs then received diphtheria toxin 0.001 cc. per kilo and survived. These dogs failed to show those changes in the size of the cardiac silhouette which have already been described in the case of the dogs which died. We have in progress experiments designed to determine whether in dogs receiving doses of diphtheria toxin large enough to cause death,

the change in the heart area is due to a change in blood volume, to blood destruction, or to change in the heart muscle itself.

Autopsies were made of the dogs, the hearts and kidneys being taken for further studies. The hearts were dissected and weighed by the method of Müller with the modifications recommended by Wilson and Herrmann. We thought it of value to do this first to complete the measurements bearing on the reduction in the size of the hearts in comparison with the body weight and second to learn whether the changes found in certain of the electrocardiograms denoted an alteration in the relative weights of the two ventricles. These studies as well as the study of the microscopic anatomy of the heart muscle and of the kidneys are not yet complete.

In addition to these methods (valve injury and diphtheria intoxication) of bringing about changes in the heart and consequently in the circulation, we design to excise portions of the kidney. In this phase of our study we think it necessary to come to definite conclusions on the relative value of various methodsof taking blood pressure in dogs by indirect means. After many experiments with varieties of apparatus applied both to the carotid artery, by the method of Van Leersum, and to the femoral artery, we have finally concluded to abandon the method of Van Leersum and to adopt the method of indirect measurement of blood pressure in the femoral artery. These methods have of course been compared with the direct method. In taking the pressure of the femoral artery we find the method of Kolls the most satisfactory. The method is essentially the method of Erlanger. There is added a record of the levels of the mercury column and a modification of the device which magnifies the excursions of the vessel wall. It may be pointed out that any method of taking blood pressure in dogs, requires careful interpretation because of the

fluctuations which are known to occur from day to day.

We are accordingly in the early stage of this study. What has so far been accomplished is in the direction of disposing of certain preliminary technical difficulties.

We have admitted patients to the hospital and have been interested in studying them from two points of view. It was shown by Cohn and Levy, that in dogs doses of digitalis presumed to be comparable to doses used in the clinic, doses which in any case do not kill intact dogs, were nevertheless sufficient to increase the degree of contraction of the ventricular muscle, when this is measured by the myocardiograph of Roy and Adami. To learn whether an effect on the contraction of the ventricular muscle occurs also in man under therapeutic conditions is a matter of importance for its own sake, but more especially since the view is commonly held that this action does not take place, that the use of digitalis in man is confined to its effect on auriculo-ventricular conduction, an effect which is seen in the reduction of the ventricular rate in fibrillation of the auricles. To test this matter of action on the muscle in man is difficult. We report now a plan we have adopted in order to test this possibility. By means of the X-ray the left border of the left ventricle can be photographed. If in a single record the excursion of this border from the diastolic to the systolic position could be photographed and measured, both in the control period and in the period when the heart is under the influence of digitalis, a comparison between the two measurements should indicate whether the drug has produced an effect. There are naturally precautions to be taken in estimating the value of such measurements, for digitalis may have an influence on cardiac rate, and alterations in rate are ., as is well known, of importance in bringing

about changes in the volume output per beat of the heart and consequently in the excursion of any given point in the cardiac surface.

In attempting to make such measurements we have followed the plan of Goett in constructing a moving X-ray camera (Fig. 1). Between the patient and the moving plate there is a lead screen. In this screen there is cut a slit 1.0 cm. high and about 17 to 18 cm. wide. By this means there is exposed to the moving plate only 1.0 cm. of the left border of the left ventricle and 1.0 cm. of the right border of the right auricle. We photograph only the shadows which pass through the 1.0 cm. slot. The exact position which is photographed in this manner is known by photographing the whole chest at the same time in the usual way, (Fig.2), this plate being placed between the individual and the lead screen. The X-ray plate is drawn upward by a motor behind the slit. The excursions of single portions of about 1.0 cm. of the two cardiac borders then appear as saw-toothed or wavy lines (Fig.3), the outer points representing the position of the portion exposed in diastole; the base of the trough its position in systole. The difference between these points is the measure of the systolic excursion of this fraction of the heart's border. In these curves can be seen the rate of change of position both of the systolic and diastolic processes at the point studied. We record both the time and the phases of respiration by photographing the movements of levers hung opposite the slot, between the X-ray tube and the lead screen. The shadows of these levers appear in the form of continuous curves. We are now making successful photographs by this method. We have as yet no data to report dealing with our problem.

A second study concerns the inability of digitalis in certain cases of heart failure in which there is edema to relieve this condition. These are cases in which the kidneys are active, for by means of diuretics,

Diagram of X-Ray Apparatus

Fig 8

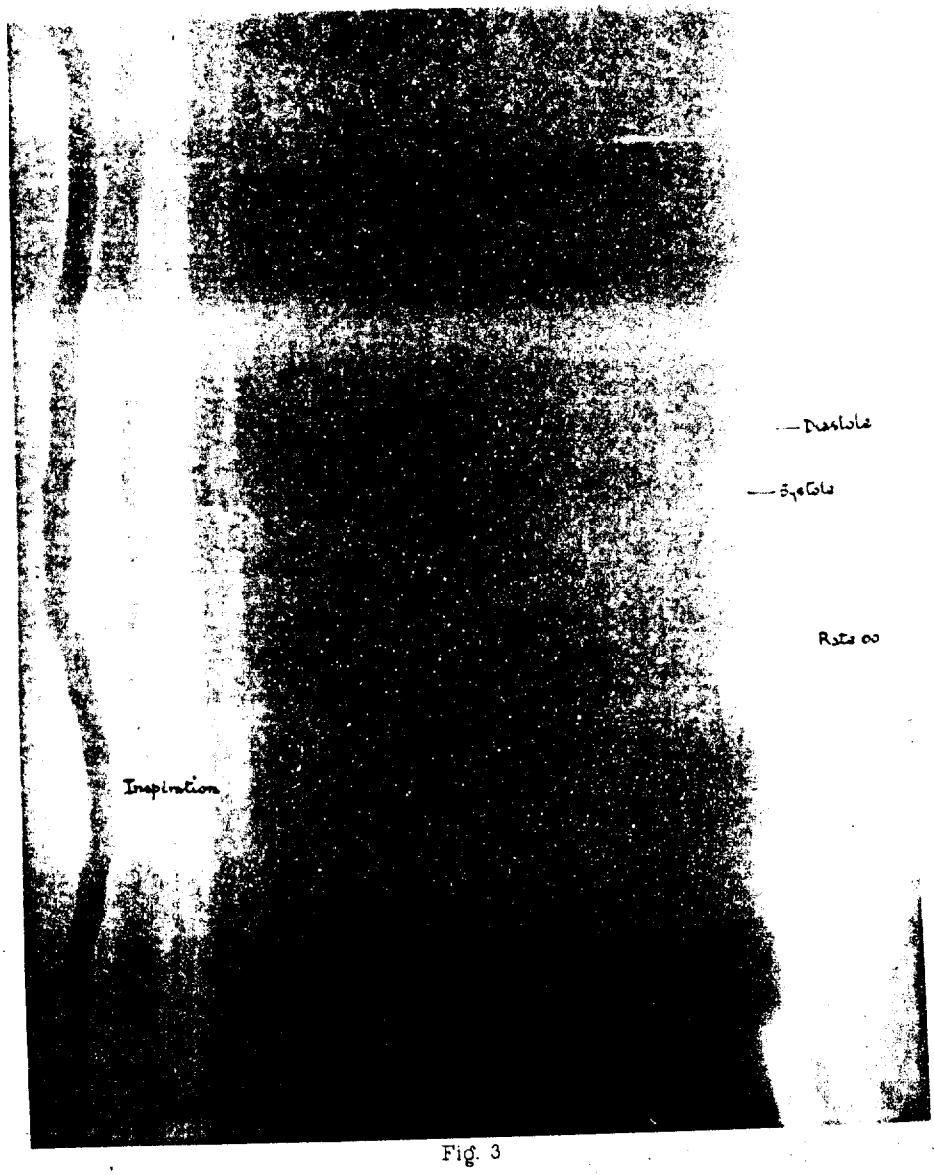

Fig. 3

such as theocin, marked increase in the output of urine is obtained though the effect is temporary; and in which the heart, or at least ... inhibitory apparatus is affected by digitalis action, as is evident by the continued effect of this drug on the rate of the ventricles when the auricles are fibrillating. The failure is apparently due to the absence of muscular action of the drug. The group of patients which corresponds to this description is large and the reasons for the failure of digitalis to affect them is unknown. In this study we are attempting to understand this group and to learn the nature of the factors which are involved.

Nephritis. (Including Report of Work Carried on in the Chemical Laboratory.)

(Report of Dr. Van Slyke.)

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

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) $\underline{L}$, the degree of oxygen unsaturation of the arterial blood coming from aerated lung areas, (2) $\underline{\alpha}$, the proportion of blood passing from the right heart to the left through unaerated channels, (3) $\underline{D}$, the oxygen consumption in the capillaries, (4) $\underline{T}$, 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 hemo-

globin content from the normal, 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

$$C = \lambda T + \frac{\alpha D}{1 - \alpha}$$

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 $\frac{\text{albumin}}{\text{globulin}}$ ratio below the normal occurred. 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 globumin in the urine of these patients is being studied with the above point in 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.

Chemistry of the Blood.

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 mano-

meter 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 measurement 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 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.

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 Harrington have found that in hemolyzed blood the same effect of oxygenation is obtained as in the solutions of pure sodium hemo-

globinate. 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.

Van Slyke, Wu, and McLean in Peking (in work reported below) showed that when the serum pH of blood is 7.40 the cell pH is only 7.26. And at 7.25 the oxygenation of pure sodium hemoglobinate increases the NaHb by only 0.62 instead of 0.68 equivalents. This accounted for half the observed difference between whole blood and pure solutions. The other half was due to some other factors, and of known differences between cell contents and NaHb solutions the most striking is the fact that all the alkali in the cells is K rather than Na (in hemolyzed blood the alkali is 2/3 Na). We accordingly determined the effect of oxygenation in solutions of potassium hemoglobinate and found the same value as in blood, when the pH difference between cells and serum is included in the calculation. The effect of oxygenation and reduction on the acid-base balance of the blood is one more physiological phenomenon which has become explainable on a physicochemical basis.

The work done in Peking was a development of the work on 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 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 H^+ ions. By some mechanism it was known that the Cl and 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 use of CO_2 tensions caused 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 solution was obtained 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.

I. For <u>electrical neutrality</u> 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 electrolytic dissociation,

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

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

II. According to <u>Donnan's law</u>, when electrolyte solutions are separated by a membrane permeable for only part of the ions, the monovalent <u>permeable</u> ions so distribute themselves that the ration 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 concentrations of osmotically active ions and molecules in each must approximate quality. We found that the unit of osmotic concentration is not the ratio $\frac{\text{dissolved substance}}{\text{volume of solution}}$ but $\frac{\text{dissolved substance}}{\text{water}}$ so that $[Cl_c^-]$ represents the equivalents of chloride per kilo of water in the cells, not the chlorides 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 B^+ 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 concentrations 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.

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

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.

It appears probable that the laws governing the distribution of salts and water between intracellular and extracellular fluids in the blood also govern the distribution in other parts of the body, between tissue cells and exudate, for example. The study is being extended in this direc-

tion, and it is hoped that it may assist in explaining the physicochemical disturbances that underlie such conditions as edema.

Acute Respiratory Diseases.

Drs. Cole, Avery, Morgan, Dahl and Stillman.

The admission of patients has not been confined to those suffering from lobar pneumonia but patients suffering from various types of the milder acute respiratory infections have also been admitted for study. Through a combination of the clinical study of these patients and more intensive laboratory investigations an attempt is being made to differentiate more clearly the various types of infection of the respiratory tract and to learn more concerning the nature of the infective agents concerned in the primary locus of infection and the mode of invasion of the lungs. Further analysis is being made of the phenomena of infection with pneumococci and the process of recovery.

Bacterial Incitant of Acute Upper Respiratory Tract Infection.

Dr. Morgan and Dr. Avery.

Studies directed toward the isolation of B. pneumosintes or other bacteria of this type from the nasal secretions have been continued. The technic followed in this study has been essentially that described by Olitsky and Gates in their investigations on the bacteriology of epidemic influenza. In all 54 specimens of naso pharyngeal secretions from 45 individuals have been examined by cultural and animal procedures. In 17 instances the washings came from the nose and throat of patients in whom the diagnosis of clinical influenza seemed certain or highly probable. The naso pharyngeal washings from 16 individuals suffering from acute coryza, sinusitis or bronchitis were similarly studied and of the remaining individuals investigated 5 were suffering from lobar pneumonia and 7 were free from respiratory infection.

From none of these 45 individuals has B. pneumosintes been recovered. On the other hand, during the study of these 54 specimens of filtered naso pharyngeal secretions, 23 strains of small Gram negative, anaerobic bacilli have been isolated. From one case of probable influenza an identical organism was isolated on four successive examinations at weekly intervals. All strains recovered thus far apparently belong to a heterogenous group of organisms which are closely allied to B. pneumosintes but are serologically distinct.

Of the 23 strains of these pneumosintes-like organisms, 13 were obtained from the 17 cases of supposed influenza; 6 strains from the 16 patients with "colds", (coryza simusitis, etc.) and 4 strains from the 7 normal individuals; from the filtered sputum and naso pharyngeal washings from 5 patients with lobar pneumonia no organisms of this type were isolated.

At present the significance of these organisms in the causation of mild infections of the upper respiratory tract must be regarded as uncertain. Their relation to each other and to B. pneumosintes is not clear. Experiments to determine their antigenic relationships are being carried out. Serological investigations are exceedingly difficult within this group because of the tendency of these bacteria to agglutinate spontaneously in normal serum and in salt mixtures.

Chemical Nature and Immunological Properties of Specific Substances of Pneumococcus Origin.

Dr. Avery and Dr. Heidelberger.

In 1917 Dr. Dochez and Dr. Avery showed that whenever pneumococci are grown in fluid media, there is present in the culture fluid, even during the early hours of growth, a substance which reacts specifically with antipneumococcus serum of the homologous type. This (soluble) substance

is demonstrable in culture filtrates during the initial growth phase of the organisms, that is, during the period of their maximum rate of multiplication when little or no cell death and disintegration is occurring. The formation of this soluble specific material by pneumococci on growth in vitro, suggested the probability of an analogous substance being formed on growth of the organism in the animal body. Examination of the blood and urine of experimentally infected animals gave proof of the presence of this substance in considerable quantities in the body fluids, following intraperitoneal infection with pneumococcus. In other words, this soluble material elaborated at the focus of the disease readily diffuses throughout the body, is taken up in the blood, passes the kidney and appears in the urine unchanged in specificity. Similarly a study of the serum of patients suffering from lobar pneumonia has revealed a substance of like nature in the circulating blood during the course of the disease in man. Furthermore, examination of the urine of patients having pneumonia due to pneumococcus types I, II and III has shown that in approximately two thirds of the cases, this specifically reacting body is excreted in the urine in quantities readily demonstrable by precipitin tests, and it has also been found that the amount of precipitable substance in the urine seems to be a measure of the severity of the infection

Sagan is Silver.

In the earlier studies by Dochez and Avery certain facts were ascertained concerning the chemical characteristics of this substance. It was found that the specific substance is not destroyed by boiling; that it is readily soluble in water, and precipitable in acetone, alcohol and ether; that it is precipitated by colloidal iron and does not dialyze through parchment; that the serological reactions

of the substance are not affected by proteolytic digestion by trypsin. Since this substance is easily soluble, thermostable and type specific it seemed an ideal basis for the beginning of a study of the relation between bacterial specificity and chemical constitution. The present report deals with the work done in this direction.

As the most abundant sources of the soluble specific substance appeared to be an 8 day old, autolyzed broth culture, this material has been used for most of the work. The organism used was the Type II pneumococcus. It was found that when the broth was concentrated on the water bath to one tenth of its volume and then treated with alcohol or acetone, a separation into two layers occurred. By addition of the precipitating agent until the upper layer no longer gave a turbidity with immune serum, the specific soluble substance could be concentrated in the lower layer, leaving in the upper layer a large proportion of the coloring matter, peptones, and other impurities, derived from the broth. In this way the active material from 75 to 100 liters of broth culture could be concentrated into a volume of about 1.5 liters. Further purification consisted in dilution and repeated precipitation with acetone or alcohol, first from the neutral solution, and finally after acidification with acetic acid. Careful fractional precipitation in the later stages resulted in the removal of inactive fractions, as it was a simple matter to redissolve a portion of each precipitate and test with immune serum. In this way it was possible in several instances, without the use of other reagents, to isolate small amounts of highly reactive material which no longer gave the biuret reaction and no longer precipitated phosphotungstic acid, one of the best precipitants for nitrogenous compounds. Under the most favorable conditions the yield was 10 milligrams of dried active substance per liter of broth used.

In other cases, however, in order to obtain products of the same degree of purity it was found necessary to precipitate the nitrogenous impurities first with phosphotungstic acid, mercuric choloride, or neutral lead acetate, or to leave them behind by precipitating the soluble substance (and other gums) by saturation with ammoniun sulfate.

The best products obtained in this way caused a precipitate to form in the homologous immune serum when the dried substance in a solution of as great as 1 part to 2,500,000 was added to the serum. On the other hand, no trace of clouding occurred when the substance, even in high concentration, was added to the heterologous serum. This concentrated and purified substance therefore is exquisitely type specific. The further study of this purified substance showed it to be free from substances giving the biuret reaction, it rotated the plane of polarized light about 31° to the right, gave the Molisch test for carbohydrates in the highest dilution at which specific precipitation occurred, (a point near the limit of delicacy for the Molisch reaction,) and yielded reducing sugars on hydrolysis with acid. The findings are summarized in the accompanying table:

Table I.

Summary of the Properties of Various Preparations of the Soluble Specific Substance of Pneumococcus Type II.

Prepn. No.	Total N. %	Hydrolysis			S o/o	P o/o	Specific Rotation $[\alpha]_D$	Pptn. with Immune serum	Meisch reaction
		NH_2 N %	NH_3 %	Red'g sugars. o/o					
4 †	6.1	3.5			1.3		Too dark	80,000	----
4 A ††	4.7			63.0		1.0	-20.6°	640,000	320,000
8 †††	2.9						+19.8°	1,250,000	640,000
9	6.6				1.8		-8.6°	640,000	------
11	2.1	0.9	1.3				+31.6°	2,500,000	1,250,000
15	2.0			49.0		0.9	+30.8°	2,500,000	1,250,000

†From urine.
††Prepn. 4 repurified.
†††From dissolved pneumococci.

Preparation 4 A was obtained from the urine of a case of Type II pneumonia, while Preparation 8 was derived from washed Type II pneumococci which had been dissolved in antiformin. It also yielded reducing substances on hydrolysis.

Since the specific soluble substance of the Type II pneumococcus appears, in its present state of purity, to consist largely of a polysaccharide, it was of interest to determine, if possible, the sugar from which the complex molecule is built up. Accordingly 700 mg. of Preparation 15 were hydrolyzed by heating 7 hours on the water bath in 0.5 N hydrochloride acid. Although the active material was unaltered by the acid at room temperature for 36 hours, heating on the water bath resulted in the disappearance of the specific reaction with immune serum, and the appearance of reducing sugars. The hydrolyzed solution was treated in the cold with phenylhydrazine and sodium acetate, and as no hydrazone separated, was then heated on the water bath. In a very short time a crystalline osazone separated. Although the amount was very small, it was possible to recrystallize the osazone. Its melting point was then 190°, showing that it was the osazone of a hexose. When dissolved in pyridine and alcohol it rotated the plane of polarized light to the left, a phenomenon characteristic of the osazone of glucose. Unfortunately the amount of substance was too small to permit the quantitative estimation of the optical activity, and conclusive proof that glucose is the sugar from which the polysaccharide is built up must await the purification of more material, which is now under way.

It will also be attempted to carry the purification of the soluble substance still further, in order to establish with certainty whether the carbohydrate reactions are due to the soluble substance itself, as now seems highly probable, or are simply due to an admixed impurity.

Studies have also been undertaken to learn more concerning the chemical and immunological properties of the protein constitutents of the pneumococcus cells. To obtain the bacterial protein as free as possible from the other constitutents of the cell, including the type specific non- protein soluble substance, the following method has been employed.

From bouillon cultures of actively growing pneumococci, the cells are removed by centrifugation and resuspended in 1/10 volume of salt solution. Solution of bacterial bodies is immediately effected by the addition of the minimum lytic amount of bile and autolysis reduced by carrying out the reaction in the cold. From the clear, slightly alkaline solution, freed from cell detritus by filtration or centrifugation the protein is precipitated by dilute acetic acid, the white floculent precipitate is washed with distilled water several times, redissolved in weak alkaline solution and again precipitated by acid. The process is repeated several times to rid the protein of the soluble substance already referred to, and the final precipitate rapidly washed with acetone and then dry ether and dried in vacuo. The product thus obtained in a form of a white powder, is readily soluble in dilute alkali and gives qualitatively all the protein reactions: Biuret, Millon, phosphorus, etc. The quantitative analysis of this substance is being undertaken as rapidly as sufficient purified material is available. The immunological study of this substance, while it is not yet complete, has yielded most interesting results. It has been found that when a solution of this substance is added to anti-pneumococcus immune serum precipitation occurs. No precipitation occurs with anti-serum produced by immunization with bacteria other than pneumococcus. It is, therefore, a specific pneumococcus substance. The remarkable fact, however, has now developed that this precipitation occurs no matter which type of anti-pneumococcus serum is employed. It is therefore, although species specific, apparently not type specific. Immuniza-

tion of animals with this pneumococcus protein substance is now under way but conclusive results have not been obtained.

These studies have resulted in observations which indicate that immunity to pneumococcus is related to two entirely distinct bacterial substances. One of them is a protein, immunity to which is very specific as regards pneumococcus but is entirely non-specific as regards type. The second substance is non-protein in nature, in its present state of purification possesses the properties of a carbohydrate, and is to a very high degree type specific. This second substance when injected alone is apparently non-antigenic. These facts have suggested entirely new conceptions concerning pneumococcus immunity which may explain a number of obscure facts, including the changes of specificity of pneumococcus with changes of virulence. The further study of this problem is now in progress.

Lungs as Portals of Entry for Bacteria in Infection in Mice.

Dr. Ernest Stillman.

In the previous report it was stated that when mice are exposed to an atmosphere containing cultures of bacteria in the form of a fine mist, the bacteria readily penetrates into the lower respiratory tract. Pneumococci which have reached the lungs as a result of this procedure usually disappear within a few hours and give rise to no infection. Attempts to cause infection to occur more frequently by means such as chilling and exposure to cold were without result. It was then attempted to lower local resistance by inducing slight injuries to the mucous membrane by inhaling ether, alcohol, and atmospheres containing finely divided quartz sand or talc in the form of dust. The procedure, however, did not increase the frequency of infection. It was found that mice could be easily intoxicated by breathing an atmosphere containing alcohol and that, if the mice,

while under the influence of alcohol, were allowed to inhale an atmosphere containing pneumococci in suspension there was a marked rise in the incidence of pneumococcus septicemia. As the direct inhalation of an alcoholic atmosphere might alter the respiratory mucosa and this might explain the increased frequency of infection, mice were intoxicated by injecting a 10% alcoholic solution intraperitoneally. When mice were intoxicated by this means and were then exposed to an atmosphere containing pneumococci in suspension there was a definite increase in the incidence of fatal septicemia, but not as great an increase as in the series of mice which had inhaled alcohol. In other experiments mice were first intoxicated by an injection of alcohol intraperitoneally; they were then exposed to an atmosphere containing B. influenzae in suspension, and then they were allowed to inhale air containing pneumococcus culture in the form of spray. In these mice the incidence of pneumococcus infection was almost as high as in the mice intoxicated by inhalation of alcohol and then exposed to the air containing pneumococci. A study of the histological changes which occur in the lungs following the inhalation of an atmosphere containing influenza bacilli and pneumococci has also been undertaken. Apparently definite pathological changes in the lungs occur as a result of the inhalation of B. influenzae.

Antigenic Properties of Pneumococci Modified by Growing in Immune Serum and by Growing in Media Containing Bile.

Dr. Dahl.

It was previously demonstrated by Miss Stryker working in this laboratory that when pneumococci are cultivated repeatedly in media containing homologous immune serum the bacteria decrease markedly in virulence and they also become much less sepcific in their immunity reactions. Dr. Avery has also shown that when pneumococci are grown in media containing minute amounts of bile the bacteria becomes resistant to the bile, and by gradually

increasing the amount of bile contained in the media the bacteria may be rendered highly resistant to the bile. The virulence of these bacteria was also found to be markedly diminished. Recently Iosnioka working with Neufeld has shown that a similar modification can be brought about by growing pneumococci at an increased temperature. It has furthermore been claimed by this observer and also by others that the immunity response in animals following the injection of these modified bacteria differs from that following the injection of virulent pneumococci. A study has been undertaken for the purpose of extending these observations and if possible of explaining the results obtained. The observations of Miss Stryker have been confirmed, and by growth in immune serum, cultures of pneumococci have now been obtained which possess almost no virulence for mice and which show little type specificity.

By repeated cultivation in media containing bile cultures of pneumococci have also been obtained which now grow readily in 90% bile. These cultures have also practically lost their virulence and type specificity.

Animals are now being immunized to these modified cultures and studies are being made of the properties of the serum. The results of these studies are not yet conclusive.

Acute Rheumatic Fever. (Report of Dr. Swift.)

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

The types of rheumatic fever studied during the past six months have been mostly of the sub-acute and chronic forms; and practically all of the patients have suffered from carditis. As mentioned in previous reports the chronic form of the disease seems to be demanding more and more of our attention.

Interesting clinical manifestations, not previously encountered by us, have been skin rashes. Four patients with skin lesions, commonly classified under the term, Erythema multiforme, have been seen recently. In two instances, these rashes have almost constantly been observed for two or three months; this we consider another indication of the persistence of the infection. In another patient the relapses have been accompanied by recurrences of the erythema. Three or four patients have had numerous subcutaneous nodules which have furnished us material for histopathological studies as well as for inoculation. We are now removing small pieces of the joint capsule and periarticular tissue for histopathological study. We have also written to various pathologists in England and in this country requesting material from fatal cases of rheumatic fever and chorea.

As far as I know, there is no comprehensive study of the pathology of rheumatic fever. The histopathology of the myocarditis is well known. The pathology of the subcutaneous nodules is fairly well studied; that of the joints during acute arthritis and of the brain in chorea patients is meagerly described. If, as we believe, all of these lesions are due to a common etiologic agent, there must be some correlation between the tissue responses in the various organs. We feel that already a more or less uniform type of response has been detected. It is probable that the animalizing of the rheumatic fever virus can only be determined by histopathological studies.

Observations on relation to leukocytosis to the activity of infection in these patients have been continued. The group of rheumatic fever patients seen in the winter of 1921-1922 has been followed this winter. Last year the patients were suffering from active infection; this year they were nearly all apparently well. Our results indicate

that when the white blood count remains consistently below 8,000 the prognosis is much more favorable than when it is above this figure. The leukocyte curve is, therefore, a valuable aid in prognosis: in the acute infection if leukocytosis persists while the patient is under the influence of anti-rheumatic drugs it serves as an indication for the continuation of these remedies even though the patient is free from symptoms; and in the sub-acute and chronic forms of the disease a persistent leukocytosis serves as an indication for continued rest, and supportive treatment. We feel that the results of our investigations along this line will be helpful to practitioners in treating rheumatic fever patients.

The clinical study of neocinchophen (or tolysin) has been continued. There is no doubt that this drug is a valuable addition to our therapeutic armamentarium, because in most instances marked antipyretic and anti-arthritic effects can be obtained with doses that are practically non-toxic. The recent announcement of Barbour and Lozensky that enormous doses of tolysin could be given to dogs and other laboratory animals without producing toxic symptoms may lead practitioners to administer too large amounts to patients. We have occasionally observed distinct evidence of renal irritation in patients; so the same care must be observed with the use of this drug as with the older and cheaper salicylates.

Dr. Boots and I have completed a series of experiments upon the subject of joint sensitization. Herry and Faber have previously claimed that the joints of animals may be rendered more sensitive to invasion of bacteria from the blood stream if the joints are previously injected with small amounts of killed streptococci or with extracts prepared from these microorganisms. The recent work of Besredka and of

Gay indicates that immunization of tissues may result from local treatment with small amounts of vaccine. This is the opposite of Harry's and Faber's point of view. Our work has failed to demonstrate any hypersensitiveness of joints injected with either vaccine or bacterial extracts. Joints so treated were about as liable to involvement as the other joints of the same animal following the intravenous inoculation of these animals with living streptococci.

Mrs. Lancefield and I have been trying to determine the presence of immune bodies against non-hemolytic streptococci in the blood of rheumatic fever patients and comparing them with similar reaction in patients suffering from streptococcus viridans endocarditis. This work is still in the preliminary stage as the methods previously developed do not seem to be sensitive enough to detect low concentrations of immune bodies, such as possibly exist in patients with the first-named disease. We are also trying to develop methods for the demonstration of antigenic substances in exudates. If such antigenic substances could be demonstrated in the arthritic fluid and pleural exudates, we would have a substance with which to test for immune bodies in the serum of patients with more doubtful conditions, and to demonstrate the unity or plurality of the rheumatic infection.

Dr. Miller and I are continuing our efforts to animalize the virus of rheumatic fever. As sources of virus we have used (1) the heart valves from a fatal case; (2) the subcutaneous nodules excised from a patient with arthritis, chorea and extensive subcutaneous nodules; (3) the joint fluid obtained from the knees of patients during the early stages of the disease. Rabbits have been the test animals. First the eyes were used; but it has been impossible consistently to obtain lesions differing very markedly from those incited in controls.

Recently, attempts have been made to carry the infection through successive inoculations of rabbits' testicles. In several instances diffuse testicular lesions have been induced in series of rabbits with material far enough removed from the original human source to insure us that tissue from a heterologous species was not responsible for the injury.

An extensive series of controls is now being observed, paralleling those inoculated with rheumatic material. While it is too early to report any conclusive results, we can state that in several instances we have obtained microscopic lesions much more severe in the "rheumatic series" than in the controls.

Chicken-pox. (Report of Dr. Rivers.)

Dr. Rivers and Dr. Tillett:

In undertaking a study of the contagious diseases occurring in children, varicella was chosen as the one with which to begin for the following reasons:

1. If produced in animals, there would be a more or less characteristic lesion to identify it.
2. There are at least three other diseases, vaccinia, variola and alastrim, with which it can be compared.
3. Relatively little attention has previously been given to the disease.
4. There is at present a great deal of interest in all diseases that produce vesicles in the skin.
5. In spite of its mildness this disease may hold the key to the understanding of more serious diseases.

The report will be made under three heads:

A. Study of vaccinia in rabbits.

B. Clinical study of cases of varicella in the hospital.

C. Results of attempts to produce varicella in animals.

A. Study of Vaccinia in Rabbits.

The study of vaccinia was undertaken in order to learn the proper

methods of handling a virus which ... the one causing varicella might be expected to resemble. It was attempted to answer the following questions: 1.- Can vaccine virus injected intravenously be made to localize in the skin by shaving and irritation? Calmette and Guerin claimed it could be done. We have confirmed their observations. 2.- Does vaccine virus get into the blood when an animal is vaccinated on the skin and, if it does, can it be recovered from the blood in demonstrable amounts? Ohtawara says the virus gets into the blood and that he was able to recover it by putting the blood of a vaccinated rabbit into the testicles of a normal rabbit and after four to six days removing the testicles, grinding them up and testing for the virus on the skin of a normal rabbit. We have confirmed and extended this work. 3.- What is the appearance of the cell inclusions in vaccinia? Rabbits' eyes were inoculated with vaccine virus and removed at different times, ranging from seventeen hours to fourteen days. Various fixatives and staining methods have been employed, including vital dyes. We are now familiar with what have been called inclusions or vaccine bodies. It seems from our observations that there are several classes of inclusions arising in different ways and that the inclusions seen under certain conditions of fixation and staining are not always the same as those seen after other fixatives and stains have been used or the same as those seen in fresh unstained cells, or in fresh cells stained with vital dyes. Conclusions from studies employing vital dyes in fresh normal tissues cannot be applied to pathological conditions, such as the vaccinated cornea. Under these circumstances diseased or dead cells are present even in the freshest specimens collected under the best conditions. For instance, mitochondria in normal cells do not stain with brilliant cresyl blue (in proper dilution) but do stain as soon as the cell dies. This dye also reacts differently to fat droplets and vacuoles,

depending upon whether the cells are dead or alive Such differences also occur when Nile blue sulphate is used. (See Lewis' article in Am. Jour. Anat.) Mitochondria, apparently undergo marked changes in the cells of a vaccinated cornea. They become swollen, vesiculated and clumped in various parts of the cell, especially around the nucleus or around certain inclusions. This has been demonstrated with brilliant cresyl blue and janus green in fresh specimens and with anilin fuchsin and methyl green after proper fixation.

B. Clinical Study of Cases of Varicella in the Hospital.

Twenty-seven patients with varicella have been admitted since the ward was opened. No instance of herpes zoster and varicella occurring in the same family at or about the same time has been noted. The clinical studies can be discussed under the following headings.

1. The blood picture.
2. Effect of trauma or hyperemia on the localization of the eruptions in the skin.
3. Allergy.
4. The skin lesions.

1.- The blood picture was followed in twenty-two cases and the findings are summarized as follows:

a. Leukopenia (below 7,000) occurred in four cases.

b. White blood cells were normal (7,000 to 9,000) in seven cases.

c. A leukocytosis (above 9,000) occurred in eleven cases. This was never marked. The highest count was 14,000; the average was between 10,000 and 11,000.

d. Twelve patients had an eosinophilia of 4 per cent or more. The eosinophiles reached 12 per cent in one instance. The increase of these cells came during convalescence. The stools were examined but no parasites or ova were found.

2.- The effect of Traum or Hyperemia on the Localization of the eruption. The localization of the eruption was certainly influenced by

trauma or hyperemia in three cases. One patient had an almost confluent eruption beneath adhesive plaster applied to the ankles to relieve the pain of a chronic arthritis. The patient had had a Schick test recently and the eruption appeared earlier at the site of injection and the vesicles were much larger and more numerous than elsewhere. In another patient the eruption was most marked beneath the napkin which gave evidence of not being changed very frequently. In a third patient, a boy who wore soft collars with his necktie drawn very tight, there was a narrow band of vesicles around his neck just beneath the tie. These observations seem to indicate clearly that the virus is disseminated through the blood stream and localizes especially where the skin is irritated. Similar observations have been mentioned casually by a few writers in regard to varicella and particularly in regard to measles by Pirquet and in regard to variola by Schick and others. Attempts were made to induce this localization after patients were admitted to the hospital by irritating the skin in certain areas. It has been impossible, however, to induce this localization under these conditions. It seems that the irritation must be present before the rash makes its appearance.

These facts may have some relationship to the occurrence of herpes zoster and varicella in the same patient at the same time. They may also have a bearing on the cases reported in the literature in which following attempts to vaccinate against varicella with fresh vesicle lymph, vesicles have later appeared at the site of vaccination. In the majority of these cases a mild general eruption appeared at the same time.

3.- Allergy. No allergic response has been demonstrated in patients recently recovered from varicella following the application of fresh vesicle fluid to the scarified skin. This may indicate that the

virus is not very concentrated in the vesicle fluid.

4.- Study of the skin lesions.

a. Vesicle fluid. 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. There are only a few cells in the fluid from young vesicles and these consist mostly of swollen epithelial cells and only an occasional giant cell. Only a few inclusions are seen at this time. Later the cells become more numerous and are of various kinds. Many are undergoing degeneration characterized by fatty or lipoidal changes and coagulation necrosis. The mitochondria exhibit a change in size, shape and location in many of the cells that show degeneration. At this time many so-called inclusions can be made out. In addition to the cytoplasmic changes, there are also profound alterations in the nuclei.

b. Skin. Small pieces of skin have been removed at various stages in the development of macules, papules and vesicles, and have been fixed, stained, and studied in many ways. The following facts are worth noting:

(1) There occurs a definite reaction around the small blood vessels in the corium just beneath the epidermis. This is probably the earliest lesion.

(2) There is a marked fatty or lipoidal degeneration of the epidermal cells as shown by sudan iii and osmic acid methods. Apparently no one has stressed this type of degeneration of the cells in the diseases in which so many cell inclusions are found, certainly not in varicella.

(3) There are changes in the mitochondria, as swelling, vesiculation and clumping.

(4) There are cell inclusions, which, after certain fixatives and

stains resemble vaccine bodies. These are not so numerous, however, as in vaccinia. Some of the granules and bodies seen in degenerating cells after treatment with certain fixatives and stains may be directly related to fatty or lipoidal changes or they may even represent altered mitochondria.

C. Attempts to Transmit Varicella.

Most of the experimental work with varicella has been limited to attempts to vaccinate normal children with fresh vesicle lymph. In the majority of instances the results have been negative or, when positive, open to criticism because of improper controls. On all previous attempts to transmit the disease to animals the results may be considered negative. In these experiments vesicle lymph was employed and the skin or eye of the animal used as the place of inoculation.

In undertaking the present attempts to transmit varicella to animals we were guided by the following considerations.

It has seemed that the virus might be present in at least one of the following locations. It might be present in the vesicle lymph, in the naso-pharyngeal secretions (especially early in the disease) in the spinal fluid, or in the blood. Experiments were therefore made with material from all of these sources and monkeys and rabbits were used for the tests. Moreover, it seemed certain that the site of injection of the infected material would be of great importance. Therefore the virus was injected into the animals in various places and in various ways. The following are some of the procedures carried out:

1. Unfiltered nasopharyngeal washings were injected intratracheally into monkeys. The skin over the abdomen was shaved shortly afterwards.

2. Vesicle fluid was scratched into the eyes and the skinof young rabbits and young monkeys.

3. Vesicle fluid was injected into the brains of young rabbits and monkeys and into the trachea of monkeys.

4. Spinal fluid from a patient with varicella was injected subarachnoidally and intravenously into a monkey. The skin over the abdomen was shaved and scarified.

5. Unfiltered nasal washings were injected into the trachea of monkeys and blood was injected into the veins at the same time. The skin over the abdomen was shaved and irritated.

6. Blood from varicella patients was injected intracerebrally into rabbits.

7. 10 cc. of uncitrated blood from varicella patients was injected intravenously into rabbits. The skin was shaved and scarified in places immediately after the injection.

In all of these experiments the results were negative or very doubtful. It seemed inadvisable, therefore, to proceed further along these lines.

Certain clinical observations previously mentioned, however, gave a clue which when followed up have yielded more successful results. It has been stated that in certain patients the localization of the lesion has strongly indicated that the virus is present in the blood in considerable concentration, at least early in the disease. Moreover, the extent of the eruption may bear no relation to the concentration of the virus in the blood. On the other hand, all the experiments previously made suggest that the virus may be in very small amounts in the vesicles.

Experiments with vaccinia have indicated that the testicles of rabbits is an excellent place for propagating a foreign virus of this type.

In view of these considerations it was decided to make a serious attempt to transmit the virus by injecting considerable amounts of blood

directly from the patients into the testicles of rabbits and to persist in the attempts to animalize the virus by repeated transfer, even though no lesions were evident on the first transfer. Blood was therefore obtained from a patient early in the disease and before coagulation occurred two cubic centimeters were injected into each testicle of a rabbit. An immediate reaction occurred and the swelling persisted and the testicles became hard and firm. After five days these testicles were removed, ground up in salt solution and 0.5 cc. to 1.5 cc. of the emulsion was injected into the testicles of other rabbits. In further transfers inoculation of the testicular material was made not only into the testicles but on the scarified skin and scarified cornea of rabbits. The results were quite striking. Lesions much resembling those of vaccinia were obtained in a number of rabbits, both in the skin and in the eye.

A considerable number of experiments similar to the above have now been carried out. Material has been obtained from five patients and positive results have now been obtained by this method with the material from three of the cases. It is evident that if the virus of varicella has been obtained it closely resembles that of vaccinia and variola.

Experiments to study the occurrence of immunity and the relation of this immunity to that of vaccinia are now under way but definite conclusions cannot be drawn at the present time. It is intended to continue this study, to learn more concerning the nature of the virus and the characteristic lesions produced. Many possible applications of this new knowledge, such as vaccination against the disease in children, are, of course, obvious. The possibility of application of a similar method in the study of other infectious diseases, such as alastrim and even measles, suggests itself.

Oxygen Therapy in Pneumonia. (Report by Dr. Binger.)

Dr. Binger and Dr. Brow.

The work on oxygen therapy in pneumonia was interrupted by the unfortunate accident in which the chamber was destroyed. Dr. Binger started at once to plan a new chamber - the plans for which are now complete. The chamber is to be built of angle iron with glass and sheet iron sides. It is to be located on the 4th floor of the hospital in room 405, formerly used for hydrotherapy equipment. A small 2 cubic/ft model of the chamber has been built and tested and found sufficiently leak tight. The new chamber is to be fire proof and as leak tight as it can be made. This should considerably reduce the cost of operation. All wiring and other sources of energy will be kept outside the oxygen rich atmosphere. This has involved numerous new problems and explains the delay in starting operations. The chamber should be completed by spring.

We have been fortunate this year in having very few cases of pneumonia with cyanosis - the ones in which oxygen therapy appears to be of so much benefit. A few cases have been treated by oxygen insufflation with a nasal catheter. This method, while it sometimes gives good practical results, does not serve as a substitute for the oxygen chamber and affords no opportunity for studying the optimum dosage of oxygen.

Experiments have been made with a bed tent constructed of rubberized canvas such as used for the construction of air-craft. This tent is easily portable and should prove to be of use in homes and in such hospitals as are unable to have chambers. The tent is not yet ready for use. The construction of it has been undertaken by the Connecticut Air Craft Company under the direction of Dr. Binger.

Acid-Base Equilibrium in Pneumonia.

Closely related to the problem of disturbed respiratory function

in pneumonia is the question of the state of the blood and the presence or absence of acidosis. Certain investigators (Means et al) believe they have demonstrated the existence of acidosis in pneumonia and have recommended the routine use of sodium bicarbonate for the purpose of increasing the CO_2 binding power of the blood. These workers constructed CO_2 absorption curves and calculated the pH of the blood by using an assumed constant. Binger, Hastings and Neill have recently published (Arch. of Int.Med. Jan.1923,) an article describing the untoward results which may follow the indiscriminate use of sodium bicarbonate. The particular case was one in which the kidneys could not excrete bicarbonate. There was retention of the salt and a concomitant and serious retention of water resulting in edema of the lungs, hydrothorax and generalized anasarca - with profound cyanosis and rapid and shallow breathing. The case was brought to the hospital in this state. Investigation of the arterial blood showed a pronounced alkalosis and anoxemia. The patient was treated in the oxygen chamber and bicarbonate administration was of course stopped. There was almost immediate recovery. The patient is now well.

This pointed to the desirability of reinvestigating the condition of acid-base balance in pneumonia by more direct methods. Hastings and Neill, Morgan and Binger have studied the arterial blood of a series of cases of pneumonia - making direct observations of oxygen and CO_2 content and combining power and hydrogen ion concentration by both the electrometric and colorometric methods. No evidence of acidosis has been found.

Lung Volume Studies.

Method: (a) The method for studying lung volume in individuals suffering from shortness of breath has been described and the report will shortly appear in the Journal of Experimental Medicine.

(b) The studies on patients with heart disease are completed and an article on this work has been submitted for publication in the Journal of Experimental Medicine. In brief the findings are as follows: The reduction in the vital capacity of the lungs observed in conditions of heart failure is generally associated with a reduction in the total lung capacity and in the so-called mid-capacity as well. No evidence of emphysema has been found - a condition described in the foreign literature. In the early cases where reduction of vital capacity is slight there is an increase in residual air - often sufficient to explain the diminished vital capacity. In the cases of long standing with marked evidence of congestion in the pulmonary circulation there is usually a reduction in residual air as well. The cause and significance of each one of these volume changes has been fully discussed and the relations which they bear to the general problem of dyspnea.

Lung Volume Studies in Lobar Pneumonia.

Dr. Binger and Dr. Brow.

The intimate relation which appeared to exist between reduction of lung volume and dyspnea indicated the desirability of studying this condition in lobar pneumonia where we know that a good deal of the air content of the lung may be replaced by solid material and where the surface through which the respiratory gases diffuse is diseased. To study a condition in which the diseased state gradually disappears and reverts to normal seemed an admirable opportunity for learning some exact facts about the pathological physiology of respiration.

Now a study of respiration in the pneumonia patient is an extremely difficult affair as any obstruction or resistance immediately varies the type of breathing. Furthermore, it was necessary to get graphic curves of respirations while the patient was breathing atmospheric

air. By modifying a large 90 Liter Tissot Spirometer and providing for continuous removal of CO_2 and supply of O_2 at the rate of consumption, it was possible to secure such tracings. This made it feasible to measure the volume of air in the lungs at any given phase of respiration. Because of the relative constancy of the expiratory position as compared to the inspiratory - lung volumes have been measured at this position i.e. the volume of air remaining in the lungs at the end of a quiet expiratory excursion is being studied. There appears to be a definite relation between this value and the type of breathing and perhaps - though this is not yet certain - to the appearance of anoxemia and cyanosis. These studies are being made throughout the course of the disease and the changes during resolution and convalescence are being noted. The work is not yet sufficiently advanced to permit of generalizations.

A series of normal individuals are being studied as well. The method has now been perfected to a very satisfactory degree of accuracy. Deviations of 100 cc. in 2 liters are maximal - representing a maximum error of 5%. The same relations appear to obtain in normals i.e. the depth and rate of pulmonary ventilation seems in some way related to the volume of air in the lung at the end of normal expiration - which, of course, is a function of the surface area of respiratory epithelium.

An effort is being made to correlate the size of the <u>resting expiratory lung volume</u> with some other physical measurement such as surface area (from the DuBois Height, Weight formula) or chest measurement - in order to be able to predict what a lung volume should be for any given individual.

<u>Report of the Pathologist</u>. (Dr. Branch)

The routine pathological work has included 9 autopsies, the

reorganization of the Museum and the running of a pathological laboratory as a unit including the training of a technician. One of the autopsies, a case of subacute bacterial endocarditis due to a hemolytic hemophylic bacillus, has been written up for publication with Dr. Miller.

Apart from the above work the chief interest has centered around the early pathological lesions of pneumococcus pneumonia.

In order to obtain some insight into the cellular reactions produced by this organism under varying conditions, a number of guinea pigs were first used. Subcutaneous injection was employed on the assumption that pneumonia is a cellulitis. By this method also dosage can be accurately measured, time periods readily taken into account, and the skin easily removed at different intervals for histological examination and impression smears. The difference of reaction in intensity per time period in immune and older animals could be readily compared with stock and young animals.

With this background we have proceeded to study the early histological lesions in the lung. Material used here was obtained from mice sprayed by Dr. Stillman in his work including those sprayed with pneumococci, alcohol and pneumococci, influenza bacillus and pneumococci, etc. Further material was also obtained from Drs. Avery and Morgan in their work on intra-tracheal injection of rabbits with nasopharyngeal washings and suspensions of infected crushed rabbit lungs. This study is still in progress.