REPORT OF THE DIRECTOR OF THE HOSPITAL

April 19, 1924

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Gentlemen:

During the past six months no noteworthy changes in staff or in the organization of the hospital have occurred. Mention was made in the last report of the diseases being studied and the study of no other diseases has been undertaken. The following is a report of the work in progress in the several departments and a statement of the results obtained.

Studies on Chicken-pox.

Dr. Rivers and Dr. Tillett.

The study of chicken-pox has been continued and extended. The results are given under the following four headings:

A.- Clinical study of cases of chicken-pox in the hospital.

B.- Further study in rabbits and in *vitro* of a virus recovered in attempting to transmit varicella to rabbits.

C.- Identification of the virus recovered in attempting to transmit varicella to rabbits.

D.- Study of vaccinia in rabbits and vaccine virus in *vitro*.

A.- Clinical Study of Cases of Varicella in the Hospital.

Since the ward was opened in October, 47 patients suffering from chicken-pox have been admitted for study and treatment.

The effects of chemical and mechanical irritation upon the locali-

tion of the virus of varicella as evidenced by the appearance of an unusual ..ber of vesicles at the site of irritation have been discussed in previous ..orts. Several striking instances of the effect of irritation upon the lo.. ..ization of the virus have been encountered this year. We have also had the ..portunity of observing the effect which a coexisting disease may have upon .. localization of the virus in varicella. There was admitted to the hospital ..ild showing various manifestations of congenital syphilis. In addition an ..ption was present, the individual lesions of which were typical of chicken-.. Most of the lesions, however, were over the face and extremities, the ..ions most involved in syphilis eruptions, and not over the trunk as is usually .. case in chicken-pox. This unusual occurrence of the varicella lesions .. parts of the body usually involved by congenital syphilis suggests strong-.. tant the syphilitic injury had caused sufficient irritation of the skin to ..fluence the localization of the varicella virus.

B.- Further Study in Rabbits and in Vitro of a Virus Recovered in Attempting to Transmit Varicella to Rabbits.

Brief history of the virus under investigation. In most of the experiments the virus which we have called Virus III was used. The lesions produced by this virus were first recognized in the fourth testicular transfer following a primary inoculation of blood from a varicella patient into the testicles of a rabbit. The virus has been transferred 75 times from rabbit to rabbit. It now acts like a fixed virus in that the injection of a small amount of the virus into the testicles of rabbits is followed in 3 to 4 days by a sharp rise of temperature to 104-106°F. Moreover, intradermal injections of the virus in dilutions of 1-1000 are regularly followed by visible skin reactions. The injection of the virus never causes death of the animal even when the injections are made into the brain. The virus, however, has been demon-

strated to be present in the blood during the height of the reaction following intratesticular and intradermal injection. This presence of the virus in the blood has been demonstrated by intratesticular injection, the presence of the virus in these testicles being determined by a positive result following the intradermal injection of the testicular material. Portions of the testicular emulsions containing the virus have been shown repeatedly to be free from ordinary anaerobic and aerobic bacteria by means of cultures on blood agar, in broth and in Smith-Noguchi tubes. Furthermore, ordinary bacteria have not been seen in stained films and dark-field preparations of the emulsions containing the virus, in stained sections of inoculated testicles or in sections of inoculated testicles impregnated with silver nitrate.

From the study of the virus in rabbits the following data have been obtained:

1. The intradermal method of inoculating Virus III gives more reliable results than those obtained by smearing the virus on the scarified skin. The development of this method has made rapid progress possible.
2. Virus III, heated 10 minutes at 55°C., will not produce visible reactions in the skin of rabbits.
3. Virus III passes through Berkefeld N and V filters. Titrations of the virus before and after filtration, however, showed that most of the virus was held back by the filters.
4. The data obtained so far indicates that the best method of preserving the virus in an active state is to filter the testicular emulsions containing the virus, add glycerol to the filtrate up to 40 per cent of the total volume, seal with vaseline and store on ice.
5. Viable Virus III produces a definite immunity in rabbits which persists for at least 6 months. The immunity follows intradermal, intratesticular,

intravenous, intracerebral or intranasal inoculations of the virus.

A single intradermal injection of Virus III which has been killed by heat does not produce a demonstrable immunity in rabbits.

No passive immunity to Virus III could be demonstrated in rabbits which had received intravenous injections of 5 to 10 cc. serum of an immune rabbit 24 hours previously.

Immune rabbit serum neutralizes Virus III either when they are mixed before injection or when the immune serum and the virus are injected into the same part of the skin at or about the same time.

Virus III and vaccine virus are immunologically distinct.

Virus III and the virus of symptomatic herpes are immunologically distinct.

Three strains of the virus under investigation are immunologically identical.

Identification of Virus III.

Virus III was recovered following the injection of blood from a varicella patient into the testicles of a rabbit and then making repeated transfers at four-day intervals from rabbit to rabbit by means of testicular inoculations. The method employed in recovering the virus, the fact that more than half the experiments which were performed in attempting to recover the virus resulted negatively, the macroscopic and microscopic lesions produced in rabbits by the virus, led us to infer that we were not improbably working with the etiological agent of chicken-pox. We realized that the final proof that the virus is the etiological agent of varicella was lacking and in our October report stated that "while the virus produces lesions in rabbits very much like those of chicken-pox, we have not shown experimentally that the virus is the etiological agent of chicken-pox." A better understanding of the behavior of the virus in animals and better methods of working with the virus were neces-

sary before we could rely on the results of experiments performed to identify the virus. The necessary methods were developed and the knowledge concerning the behavior of the virus in normal and immune animals was obtained as has been described above. With this information it was possible to undertake the identification of Virus III. The results of this work follow:

(1) The first step has consisted in determining whether or not the intravenous injection of serum and whole blood from convalescent chicken-pox patients will protect normal rabbits against the virus. It has been found that 10 cc. of serum or whole blood of patients convalescent from chicken-pox when injected intravenously will not protect normal rabbits against virus inoculated intradermally 24 hours after the administration of the serum or blood

This might have been expected, however, since, as we have stated, the serum of immune rabbits injected intravenously into normal rabbits will not protect the latter against intradermal inoculation.

(2) Since immune rabbit serum neutralizes Virus III in vitro and since a reliable technique has been devised by which this neutralization can be demonstrated, it seemed wise to determine if the serum of patients convalescent from varicella possesses any demonstrable virucidal properties for Virus III, or if the serum collected during convalescence neutralizes more virus than does that collected during the first two days of the disease. The sera from two normal adults and from fourteen patients convalescent from varicella were studied and were found to have no demonstrable neutralizing effect upon Virus III in vitro. Furthermore, in four instances no differences could be detected between the serum collected during the disease and the serum collected from the same patients during convalescence. The serum from patients convalescent from varicella, therefore, does not neutralize Virus III in vitro. The possibility however remains that the virus in the tubes employed may be too concentrated or too active to be affected by the small amount of immune

bodies that may be present in the serum of convalescents.

(3) It is known that the virus is present in small amounts of blood taken from a rabbit 4 days after intratesticular inoculation with Virus III and an active immunity promptly appears in rabbits inoculated with small amounts of this blood. Furthermore, there is evidence that the blood of varicella patients contains the etiological agent of chicken-pox. It was therefore decided to determine if animals could be actively immunized against Virus III by previously inoculating them with fresh blood obtained from patients early in the course of the disease. In a few animals instead of inoculating with patients' blood vesicle fluid and nasal washings were injected. After the inoculations the animals were kept 21 to 67 days, and then intradermal inoculations of various dilutions of active Virus III were made in order to determine whether or not the animals were immune. In each experiment the activity of the virus was tested by inoculating at the same time known normal and immune animals. Eleven experiments were performed in which 39 test and 23 control animals were employed. The percentage of immune rabbits was found to be the same in the control as in the test animals, 26 per cent. Three of the experiments taken alone might be interpreted as evidence that the injection of blood from chicken-pox patients into animals produces immunity against Virus III, but when all eleven of the experiments are considered together, the evidence does not support such an idea.

The high percentage of immune animals (26%) found in our control series in these experiments, while we had previously found only 15% of stock rabbits refractory, may possibly be explained by the occurrence of cross infection in the cages.

The control animals in experiments 2, 3, 5, 6, 9 and 10, all received blood from the same normal man. All four control animals in experiment

9 were immune and only one of the twelve rabbits controlling the other experiments was immune. Now the four control animals in experiment 9 were kept in the same cage 54 days before testing. An analogous observation was made with the test animals in experiment 3. Three animals in this experiment which were kept in the same cage for 25 days were all found to be immune on testing. It is obvious, therefore, that in making experiments with this virus to determine the development of immunity care must be taken to avoid cross infection. This is of special importance where positive conclusions are reached. This possibility has been carefully considered in all of our later experiments.

It has been thought advisable to ascertain before each experiment was undertaken whether or not the animals to be tested were susceptible to infection or were resistant since, as we have stated, 15% of all stock rabbits are immune. It is impossible to do this directly since it is obvious that the test for immunity by skin inoculation itself confers immunity. Testing the power of the normal blood obtained before the experiment to neutralize the virus in vitro and comparing this with the neutralizing power of the blood of the same animal obtained later is of considerable significance and this method was employed in the later experiments. This method does not always give absolutely conclusive results, however, since inability of the blood to neutralize the virus does not always indicate a lack of immunity.

Inoculation of Active Virus III into Animals other Than Rabbits.

Guinea pigs. Two guinea pigs received intradermal inoculations of active Virus III. No visible reaction appeared in either pig during three weeks of observation.

White mice. Six white mice received intraperitoneal injections of 0.25 to 1.25 cc. of active Virus III. During 10 days of observation all the

...ice remained well and active.

Monkeys. Each of two monkeys (Macacus rhesus) received an intra-...nous injection of 2 cc. of active Virus III in 8 cc. of physiological salt ...lution, and an intradermal inoculation of the same virus. No visible skin ...ctions, no significant changes in the temperature or in the blood currents ...curred in either monkey during 3 weeks of observation.

Humans. Two men, physicians, volunteered for intradermal inocula-...ions of active Virus III. One of the men had varicella in childhood, the ...r had never suffered from varicella. Each volunteer received 0.2 cc. of ...tive virus intradermally on the left upper arm. The volunteer who had not ...ffered from varicella experienced no general reaction and only a mild local ...e consisting of redness and tenderness in the immediate vicinity of the in-...culation which disappeared entirely in 3 days. The man who had had vari-...lla in childhood experienced a more severe reaction. Eight hours after the inoculation a chilly sensation, headache, backache and general malaise ...ere noticed. The arm at the site of the inoculation became red, swollen, tender and painful. The general reaction disappeared after 48 hours. The local reaction increased in intensity for 36 hours; the redness, swelling and tenderness extended half way down the forearm. The axillary glands were swollen and tender. The local reaction gradually subsided and disappeared in 5 days. In neither of the subjects did a vesicle or an open lesion appear at the site of the inoculation. No generalized eruption appeared in either man. Both individuals were still well a month after the injections. The blood serum of neither man neutralized Virus III in vitro before the inoculations and is now being studied at weekly intervals for the occurrence of virucidal properties.

Interpretation of the Results of our Work with Virus III.

From the work reported previously and that presented in this report

it is evident that an active, transmissible agent is being studied which partakes of the characters of the so-called filterable viruses. By immunological tests in humans and rabbits it has been impossible to obtain any evidence that varicella patients are suffering or have suffered from infection with this virus. On the other hand, the studies do bring evidence that we are dealing with a specific previously unknown filterable virus which is quite distinct from vaccine virus and the virus of symptomatic herpes. There is no evidence that Virus III is the etiological agent of snuffles. The virus of infectious myxomatosis is the only filterable virus indigenous to rabbits concerning which we have been able to find reports in the literature. This virus was first reported by Sanarelli and further described by Splendore and Moses. It produces fatal myxomatous tumors on rabbits, is filterable and can be transmitted indefinitely from rabbit to rabbit. Whether the virus studied by us is an unknown virus of human origin, is a virus indigenous to rabbits, or possibly an agent of a peculiar character not hitherto studied must be left undecided for the present.

Study of Vaccinia in Rabbits and Vaccine Virus in Vitro.

Filtration of Vaccine Virus Through Berkefeld Filters. There has always been some doubt as to whether vaccine virus is really filterable. Negri and a few other workers apparently found it to pass through a filter. Many investigators, however, have been unsuccessful. Our experiments seem to show conclusively that vaccine virus passes through Berkefeld filters V, N and W. Although some of the virus passes the filters, the greater portion of it is held back by them. If the filtrates are inoculated on the scarified skin or cornea no evidence of the presence of the virus will be obtained. If large amounts of the filtrates are injected into the testicles of normal rabbits, however, the virus multiplies and when the testicles are removed, emulsified

and smeared on the skin of another rabbit confluent eruptions typical of vaccinia appear. We have not confused this with Virus III.

Acute Rheumatic Fever.

Dr. Homer F. Swift, Dr. Andrewes and Dr. Miller.

The clinical material for the study of rheumatic fever this winter has been satisfactory, as many more applications for admission have been received than we could accommodate. Three more strictly clinical problems are under investigation: I. Methods of administration of sodium salicylate. II. Excretion of salicylate in the cerebro-spinal fluid. III. Alteration in size of the heart as disclosed by X-ray pictures.

I. The question as to whether the sodium salicylate is less toxic when administered with sodium bicarbonate has never been satisfactorily answered. We are, therefore, determining in each patient the toxic dose both with and without bicarbonate and altering the order in which the single and combined drugs are given. One difficulty encountered is that the toxic dose in an individual differs at different periods; when he is febrile he is usually more susceptible to toxic drug action than after recovery from the acute stages of the disease. While the results so far obtained are suggestive that the simultaneous administration of sodium bicarbonate renders the salicylate less toxic, final conclusions must be reserved until further work along this line is completed.

II. Becuase of the intimate association of chorea minor and rheumatic fever it is desirable to know whether the salicyl ion - which seems to be the active agent against certain exudative symptoms of rheumatic fever- passes over from the blood into the cerebro-spinal fluid; for if it were not so excreted it might explain in part the comparative resistance of the symptoms of chorea to the salicylates. Dr. Andrewes has examined the fluid of eight

patients under the influence of salicylates and as a control that of about fifty syphilitic patients. In none of the latter control group was any salicyl found; but since the technique has been perfected salicyl has been detected in the fluid of all of the last six salicylate treated patients. This work is being continued.

III. With the information available that over 95 per cent of rheumatic fever patients have electrocardiographic evidence of deranged cardiac function, it became desirable to determine whether there was a corresponding alteration in the size of the heart. Two meter X-ray pictures are taken at frequent intervals, and the total size as well as the various diameters of the heart as recorded in the film are charted so as to compare them with clinical symptoms and electrocardiograms. In several cases the change in the size of the heart shadow has been quite marked. We hope the results of these studies will be available when the group of patients now in the hospital has been more completely followed.

Mrs. Lancefield and Dr. Swift are continuing the investigation concerning the appearance of antibodies against nonhemolytic streptococci in the blood of rheumatic fever patients. As noted in the last report they were unable with any of the methods previously recommended to obtain an antigen that was sufficiently sensitive to detect complement binding antibodies in the blood of all patients suffering from Streptococcus viridans endocarditis. But with nucleo-proteins prepared from four different strains of nonhemolytic streptococci and combined in a single solution they have been able to obtain positive reactions in seven consecutive cases of Streptococcus viridans endocarditis - all that have been available. The antigen, however, is so sensitive that with it the serum of practically all syphilitic patients tried gives positive reactions. It is interesting that this streptococcus nucleoprotein gives reactions with syphilitic sera of almost the same intensity as

as obtained with the usual lipoid reagents used in the Wassermann reaction. rom the standpoint of specificity for streptococcus infection, therefore, ur antigen is too sensitive. Its availability lies in the fact that as far s we have gone it detects antibodies in the blood of all patients suffering rom known nonhemolytic streptococcus infections. If rheumatic fever in ll instances is due to nonhemolytic streptococcus infections one would expect the blood of patients to give positive reactions with an antigen of this type. oreover, if the arthritis were a hypersensitive phenomenon similar in nature to that of serum disease one would expect to detect these antibodies at the time of the onset of arthritis or shortly afterwards, for in serum disease precipitins against horse serum are usually detectable at the time of or shortly after the appearance of arthritis. On the other hand, if the recovery from rheumatic fever were an indication of the establishing of immunity against nonhemolytic streptococcus, one would not expect to detect antibodies until the patient was well on the road towards recovery. In order to test these two hypotheses we are examining the blood of our patients every 1 or 2 weeks during their stay in the hospital and at longer intervals after discharge. 19 patients with active rheumatic fever have been examined: 12 of these had negative reactions at the time of admission; 4 had weakly positive reactions - too slight to be considered significant; and 3 had strongly positive reactions. 4 of the patients with initial negative reactions have later developed positive reactions: in 1 instance moderately and in 3 instances strongly positive. These preliminary figures are subject to alteration as more cases are followed over longer periods.

At the same time controls consisting of well persons, and patients suffering from pneumonia, nephritis, and chronic cardiac disease are being followed. It is of interest to note that almost as high a proportion of well

persons have shown positive reactions upon first examination as was shown by the rhuematic fever group. This is not surprising when one considers the frequency of streptococcus infections of the upper respiratory tract.

In order to make this work of value in throwing some light on the relation of streptococci infections to rheumatic fever all groups of patients and controls must be repeatedly examined at frequent intervals over long periods. This is very time consuming, but in view of the evidence already obtained it is felt worth while to follow the problem to its logical conclusion.

Drs. Andrewes and Miller, assisted in part by Dr. Swift, have devoted the major part of the winter to attempting to cultivate the hypothetical virus of rheumatic fever in the testicles of rabbits by means of certain modifications in the technique proposed by Rivers and Tillett for the cultivation of the virus of chicken-pox. Last year Dr. Miller and Dr. Swift were able at times to produce lesions in rabbits' testicles by inoculation with material obtained from rheumatic fever patients. These lesions were disseminated focal perivascular collections of cells in the interstitial tissue, and were not unlike those seen in the tissues of rheumatic fever patients. The inoculations were made at intervals of from 3 to 4 or 5 weeks. With the technique then used we were unable to carry on the series continuously. In several control series the results were entirely negative; but in one series, in which the original material inoculated was from the heart valves of a normal dog, lesions were obtained in some of the rabbits' testicles not clearly distinguishable from those in the series from rheumatic fever patients.

While these findings were suggestive that a virus might be obtained from rheumatic fever it was evident that with the technique used no conclusive results were obtained.

It was, therefore, thought advisable to attempt to depress the rabbit's resistance by one of two methods before inoculating them: (1) by X-raying the animals; (2) by treating them with benzol. As leucocyte studies in our patients indicate that there is a leucocytosis made up largely of polymorphonuclear leucocytes, it was thought that depression of these elements in rabbits with benzol might render the animals more susceptible to the virus of rheumatic fever. The transfer of supposedly infectious material was to be carried out every four days as recommended by Rivers.

Twelve series of animals have been inoculated as follows: six with blood, one with throat washings, and five with joint fluid.

In the series carried the longest, 21 generations, definite microscopic lesions appeared in the third generation and macroscopic lesions in the fourth and fifth. When the virus has reached a certain degree of virulence or concentration the animals have fever, and swollen testicles on the third to fifth day; and on removal from the body the testicles are swollen, edematous and hemorrhagic. When the virulence is less marked there may be no clinical symptoms and the testicles show only slight macroscopic changes; but on microscopic examination there is marked alteration to complete inhibition of spermatogenesis and numerous interstitial lesions made up of mononuclear cells and polymorphonuclears.

On several occasions the so-called F-R strain has shown evidences of increased activity alternating with periods of decreased virulence in which evidence of continuation of activity was chiefly histological. In another strain, Cl. A, hemorrhagic lesions have been much less in evidence but characteristic microscopic lesions were constantly present throughout the inoculated series.

Constant bacteriological control has failed to reveal any common bacteria - either aerobic or anaerobic - and no bacteria have been seen on

examination of the sections.

The virus has been brought to light in three, and perhaps five, different series. Two series of animals run at the same time as the positive series have shown no lesions and may be considered negative. No series of animals, however, have been inoculated with blood from nonrheumatic fever patients, nor with testicles, nor blood of normal rabbits. This point will be discussed later.

We were able to show that the virus causing the lesions resists treatment with 50 per cent glycerine; it also can be kept at practically its original strength by freezing and drying the tissue according to the method elaborated here four years ago for preserving bacterial cultures.

In animals inoculated with the F-R strain it was evident in January that the clinical course and gross lesions were very similar to those obtained by Dr. Rivers with his supposedly chicken-pox virus. We have also found in the lesions intranuclear inclusion bodies similar to those found by him and also similar to those described in the lesions of animals inoculated with herpes. The material inoculated intracutaneously has produced areas of erythema after 4 to 6 days; and inoculated into the mediastinal region has caused a fibrinous pericarditis and diffuse myocarditis; injected into the knees of rabbits it produces a mild arthritis. Intranuclear inclusion bodies were found in the pericardial and myocardial lesions, and in the synovial fluid from an inflamed joint.

From this point Dr. Rivers and we have been working together to determine whether the virus obtained by him, presumably from chickenpox patients, and by us, supposedly from rheumatic fever patients, was the same. As the two series of animals have been kept separate since inoculation it would seem that they serve as good controls of one another, because spontaneous cross infection was highly improbable. Dr. Rivers had found that

.is animals may be immunized to his virus by intranasal inoculation; he feels, .erefore, that the virus might be easily transmitted from infected to .al animals by close contact or by careless handling on the part of .tendants.

In order to prove the identity of the virus in the two series we .e infecting animals in our laboratory, keeping them for two weeks or more .d then giving them to Dr. Rivers to test for skin immunity to his virus. .is reciprocating in a similar way with his animals. He has shown that .. blood of his previously infected animals neutralizes his virus so that .reaction is produced when the mixture is inoculated into the skin of .mal rabbits. We are studying the ability of the serum from his immune .mals to neutralize our virus and the ability of the blood of our immune .mals to neutralize his virus. The results obtained so far indicate that .e two viruses are probably the same.

We are in the midst of a study upon the ability of the serum of .heumatic fever patients to neutralize our virus; and upon the filterability .f our virus.

If - as now seems probable - the two viruses are identical, and if the serum of rheumatic fever patients does not neutralize them it seems evident that the strains of virus we have obtained are not causative agents of rheumatic fever. It remains then to determine whether this virus can be obtained from normal rabbits or from febrile patients suffering from some disease of known etiology; for the possibility must be kept in mind that the virus is of human origin, and is comparatively innocuous for humans, but may invade the blood stream while the patient is febrile from other causes. Dr. Rivers is studying the nature of the virus, its immunological reactions and its relation to the virus of herpes, vaccinia, etc. He feels strongly that controls should not be run in animal rooms in which rabbits known to be infect-

ed are kept. We, therefore, propose to clean out our animal room entirely, have it thoroughly disinfected, start with a new stock of rabbits and run several series of controls in an attempt to determine whether the virus is of human or rabbit origin. With our present knowledge this information can be much more readily and rapidly obtained than would have been possible a few months ago.

During the investigation we have had the closest cooperation with Dr. Rivers: and only because of the information furnished by him and learned as a result of two years of his work have we been able to make progress so rapidly, and to determine quickly the relationship or lack of relationship between the disease in rabbits and rheumatic fever.

Even though our work has not disclosed positive information as to the etiology of rheumatic fever, it has served as a control of Dr. Rivers' findings which he thought inadvisable to attempt in his own laboratory, and has served to confirm his recently developed opinion that the virus first suspected of being the cause of chicken-pox has in reality little to do with that disease. The combined work in attempting to isolate the etiologic agent of varicella and rheumatic fever has disclosed a new virus; one that must be considered when the method of inoculating rabbits is used for the purpose of studying diseases of unknown etiology.

Studies on Physiology and Pathology of the Circulation.

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

Our work has in general continued in the direction that was described last year. The experiments that have been carried on were designed to illuminate problems having to do with the behavior of the heart muscle, notably with the function of contraction. The study of this function can be pursued in so many ways that it was thought best to regard it from a single

angle, namely, from the point of view of its change with time. There are several reasons for this choice both on account of problems in physiology and those in the clinic. Aside from the injuries which it may suffer as the result of infectious diseases, the heart appears to undergo changes with time as do other tissues and organs of the body. It is these changes which we are investigating. These have a great interest not only on their own account, but also because with them are associated the ability to compensate for injury by the mechanisms of hypertrophy and presumably of hyperplasia. To find suitable organisms in which to study these changes in a satisfactory manner is difficult. But an insight into certain of the changes which take place may be obtained in a period of rapid growth such as is illustrated during embryonic development. The embryonic period has the advantage moreover, especially at this stage of our study, in that the conditions and factors of incubation can be adequately controlled. On account of the experience already acquired with chicken embryos and because this seemed a distinctly suitable organism, our studies have been continued with this species. Our source of supply of eggs remains the same as last year and is quite satisfactory.

This year it has been possible to resume the study on the heart beat of the chicken embryo which was interrupted last year. The attempt was made then to record the rate of the heart at succeeding ages by the galvanometric method. The method itself was quite feasible and offered, as other investigators had formerly found, no serious difficulties in obtaining electrocardiograms. But there were errors in this technique due to two sources; the first was due to the mechanical stimulus which applying the electrodes offered to the embryo, and the second to chemical alterations which undoubtedly resulted from the application of electrodes necessarily moistened with salt solution. It was noticed during the course of these experiments that when a

small opening had been cut into the shell, it was possible to learn the heart rate either by counting the pulsations of an artery or in younger embryos by counting the pulsations of the heart itself. At this point, as has been said, the experiments were interrupted. For in order to proceed satisfactorily not only with this phase but with other associated experiments, it was thought better to wait for the completion of the construction of a constant temperature room.

The constant temperature room was built and the tests completed during the autumn (1923). It was built with the advice and help of Mr. E. B. Smith. It is a pleasure to record that the result is eminently successful. A temperature of 38.5°C has been maintained in the room and its constancy has been controlled by recording apparatus. For weeks at a time this temperature remains constant. When two persons work in the room, when an electric lamp with resistance and also a Bunsen flame are in operation, the temperature rises 1½ to 2 degrees, but this elevation is easily neutralized by increasing the ventilation and the exhaust. The ventilation is accomplished by means of the common compressed air service, the use of which affords an added advantage in that the air is dry, giving a humidity to the room of about 25 per cent. We anticipated the low humidity and therefore built in a tight water-jacketed box in which the eggs are incubated and in which the atmosphere can be saturated with moisture above that of the rest of the room. Daylight enters the room through a large double window. To work in the room is quite comfortable, certainly for periods of three to four hours. Its advantages so far as maintaining the temperature of embryonic tissues constant during operations are of course obvious. The room with its incubator make the factors having to do with incubation, constancy of temperature and humidity, easily controllable. The importance of this in that it results in being certain of the age of embryos is naturally clear. How important it has been in other

connections will be mentioned presently.

In this room then the experiments of the rhythm producing functions of the heart and the changes of these with time were pursued. So far, the number of experiments which have been performed is insufficient to permit presenting the data obtained in a comprehensive fashion. Of those done on embryos after the 4th day of incubation it appears that, as a rule, the rate is 200 beats per minute or over; before this time, on the 2nd and 3rd days, the rate appears to be considerably lower. Whether the rate curve will ultimately bear a relation to "The Curve of Potential Growth" reported formerly, cannot yet be foretold; but to find such a relation would not be surprising. The bearing and importance of such a finding on metabolic occurrences in the embryo, presently to be mentioned, and on questions of energetics of the muscle, the study of which is projected, need not be emphasized.

After the rate of the heart has been ascertained the next step in the experiment is concerned with ascertaining when differentiation of its parts takes place and with learning the function of these parts. The embryo is taken from the egg and placed on a slide in enough chicken serum to cover it. With the aid of a dissecting microscope the heart is excised. Its age is next identified by comparing its morphology with a series of drawings prepared for this purpose last summer. It is then drawn with an Abbe drawing apparatus, the magnification being recorded. The area of the drawing is subsequently measured. The heart is then dissected. The following appears to be the best plan, especially after the 4th day. The great vessels are ablated and discarded. Then in the following order the fragments are cut off and planted in a medium consisting of adult chicken plasma together with a minute amount of tissue extract taken from embryos of the same age as those from which the fragments are derived (see diagram): (1) The tip of the right auricle; (2) a fragment lying mesial to it and of about the same size;

(3) the tip of the left auricle; (4) a fragment of the left side corresponding to 2; (5) the central portion, consisting presumably of the interauricular septum and the anterior and posterior walls adjacent to it; 6 to 11 being fragments of the ventricle. The cross hatched piece is discarded since it represents primary or secondary septa; the borders are taken as representative presumably of the primitive cardiac tube. The location of all the fragments is recorded on the drawing made at the time with the Abbe apparatus. A drawing from an actual experiment (No. 30) is reproduced.

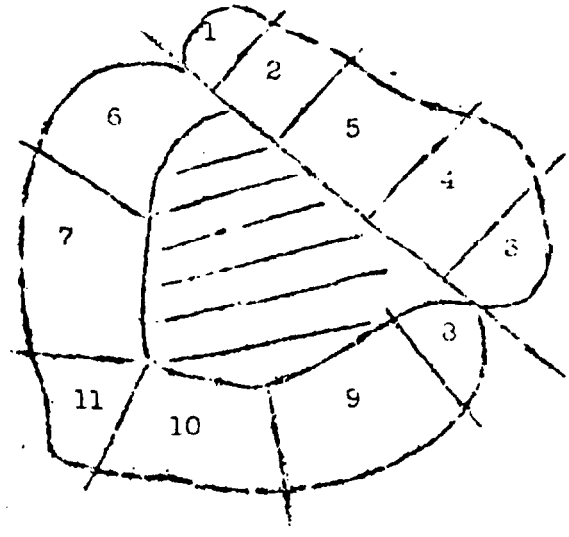

Table 1.
Experiment 30.

		Rate Highest	Rate Average	Area sq.cm.
Embryo 5.8 hrs. +			204	3.36
Fragment 1	auricles	56	49	0.055
" 2		42	39	0.061
" 3		46	35	0.092
" 4		52	43	0.092
" 5		200	194	0.188
" 6	ventricles	34	29	0.155
" 7		60	42	0.096
" 8		56	46	0.066
" 9		36	28	0.159
" 10		48	43	0.118
" 11		56	45	0.129

When the heart is less than 3 days old the procedure is illustrated

in the accompanying drawing (Experiment 29).

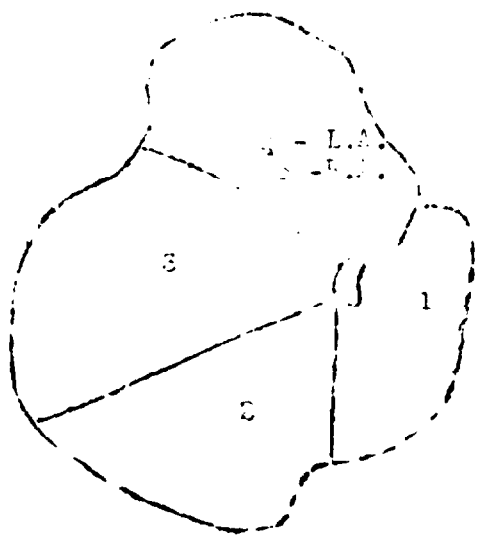

Table 2.
Experiment 29.

	Rate	Area in sq. cm.
Intact embryo 30 days +	146	0.67
Fragment 1 (vessels)	0	0.111
" 2	37	0.122
" 3	62	0.155
" 4 (left auricle)	138	0.104
" 5 (right auricle)	143	0.100

The following facts are shown in the table: (1) The size of the fragments is fairly uniform, but that even when not quite uniform, such differences in size result in no demonstrable difference in performance. In Experiment 30 fragment 1, is one third the size of fragment 6, but its rate is higher. Fragment 1 is auricular however, whereas fragment 6 is not. (2) In Experi-

ment 29 the rate of the two auricular fragments are about equal. That is to say no portion of the auricles has taken on yet a pace making function. (3) The rate of the auricles is higher than that of the ventricles, that is to say, already on the third day a differentiation in the rate producing function between auricular and ventricular end of the cardiac tube exists. (4) In Experiment 30, a differentiation has taken place among the five fragments of the auricles. It is the central portion (fragment 5) alone which attains a high rate and makes the pace for the whole heart. (5) The rate of the four other fragments is much less than that of the pace-maker. (6) The rate of the ventricular pieces is approximately uniform and about equal to that of the auricles. (7) the rate of the highest auricular fragments in both hearts equals that of the respective intact heart.

The description given of these two experiments is representative of those so far performed between the ages 3 to 10 days. Before and after these ages conspicuous deviations seem to prevail. It is perhaps better to postpone a description of these until more data have been accumulated. Enough has been said to indicate that much remains to be learned of changes which take place in time in a single portion of the life cycle of a tissue like heart muscle and that information of this sort is desirable in a consideration of its dynamics and energetics to which these studies lead.

In the last report an account of certain growth experiments was given. It was shown that the growth rate of the chicken embryo as a whole as well as fragments taken from the ventricles of the heart decreased progressively with age.

In studying the changes with age involved in the growth and functional differentiation of chicken embryos it was deemed necessary to investifate and standardize their environment. The environment included (1) the food supply of the embryo, namely, yolk and albumen and, as these are influ-

enced by outside conditions, and (2) the atmosphere of the incubation chamber. The latter studies on the conditions of incubation which should have been conducted at the beginning of our investigation were postponed until a constant temperature room had been constructed.

Of the variable factors involved in the development of fertile eggs temperature and humidity are of paramount significance. The results of our studies show that the composition of the whole egg and the chemical energy lost during incubation as well as the growth rate and constitution of the embryo itself are dependent in a large measure upon these two factors. Previous investigators have differed about such questions as the amount of weight lost by an egg during incubation and its water and fat content. With the information now at hand it can be surmised that their disagreements were due to the fact that no quantitative estimations were made of the conditions under which their eggs were incubated.

Our data on incubation variables is almost complete. The rate of ontogenesis is determined to a large extent by the temperature, whereas the concentration of water and fat in the egg is a function of the humidity. Incubated eggs lose a constant amount of weight each day, the quantity lost depending upon the concentration of water vapor in the atmosphere. The weight lost by each egg during incubation may vary from 1.0 gm. when the humidity is 92 per cent, to 10.0 gm. when it is 28 per cent. Despite this loss of weight the concentration of water in the fertile egg remains the same, that is to say, approximately, 75 per cent. It appears that 25 per cent of the weight lost during incubation must be derived from solid substance eliminated as the carbon dioxide and water of metabolism. We are attempting to find by analyses whether the burning of fat in the yolk may be held entirely accountable for this 25 per cent deficiency. Tangl has proposed a theory widely

quoted in the literature which states that the fat burnt during incubation measures the energy necessary for ontogenesis. If this were true it would be of fundamental significance. The analyses made here show, however, that the amount of fat lost is a function of the humidity. This mechanism seems to be significant for preserving a constant concentration of water in the egg. Ontogenesis however progresses at a uniform rate regardless of the amount of fat katabolism. We are accordingly satisfied in regard to the effect of the more important variables in regulating the growth of the embryo at least to a degree of exactitude sufficient for our purpose. With these data it has been possible to establish standard conditions which can be reliably maintained in the constant temperature room and in this manner enhance the probability that every incubated egg is developing at approximately the same rate. The variations found in the functions analyzed, such as growth, chemical constitution and heart rate, are not, we believe, the result of disturbances in the environment of the egg.

Having established uniform conditions we are repeating some of our earlier experiments on the growth rate of the embryo as a whole so as to reassure ourselves as to their validity in a more or less stable environment.

At present we are principally engaged in chemical studies to show the changing constitution of the embryo with age as compared with its yolk and albumen, whence it derives its nutrition. Analyses for water, proteins, fats and chlorides at various ages are almost complete. It was necessary to analyze the egg as a whole because we wished to compare the chemical constitution of the embryo with its environment, expressing this as a ratio or dynamic equilibrium shifting progressively with age.

The curve for the concentration of total solids with age is S-shaped. It seems therefore that dehydration cannot be the chemical bases for the negative

acceleration of growth. The curve showing the latent period of tissue cultures as a function of embryonic age, however, is not dissimilar to the water curve. That the two are related is suggested by the fact that dilution of the medium with Ringer's solution decreases very markedly the prolongation of the latent period which is characteristic of differentiation with age. We hope by this method of comparing rate curves to find the chemical basis of other ontogenetic processes. (Chart 1.)

In these chemical studies it is our purpose to analyze (1) the more important substances regulating osmotic conditions on the one hand, such as water, protein, bicarbonate, chlorides, total bases and the hydrogen ion concentration; and (2) the substances of fuel value to the embryo; namely, fats, proteins and carbohydrates. We had intended to investigate the gas exchange through the shell, but it appears that this problem is now being studied in the United States Department of Agriculture. We hope to be able to avail ourselves of the data obtained there.

These chemical studies are we believe destined to be useful in interpreting changes in mechanism which occur with age in the dynamics of heart muscle and in cardiac efficiency. In addition to this aspect of the study, they may conceivably contribute to more general conceptions having to do with the phenomena associated with various age periods.

With Dr. Stewart the effect of digitalis in therapeutic doses on the contraction of heart muscle has been studied by means of X-ray curves obtained by the use of the X-ray moving film apparatus, the description of which was incorporated in a previous report. Few cases have so far been examined because of the anatomical requirements which it is desirable to meet. We prefer patients who are thin chested and in whom there is a fairly wide space of lung tissue between the shadow of the heart and that of the chest wall; the heart must be, in other words, not too greatly enlarged. In such

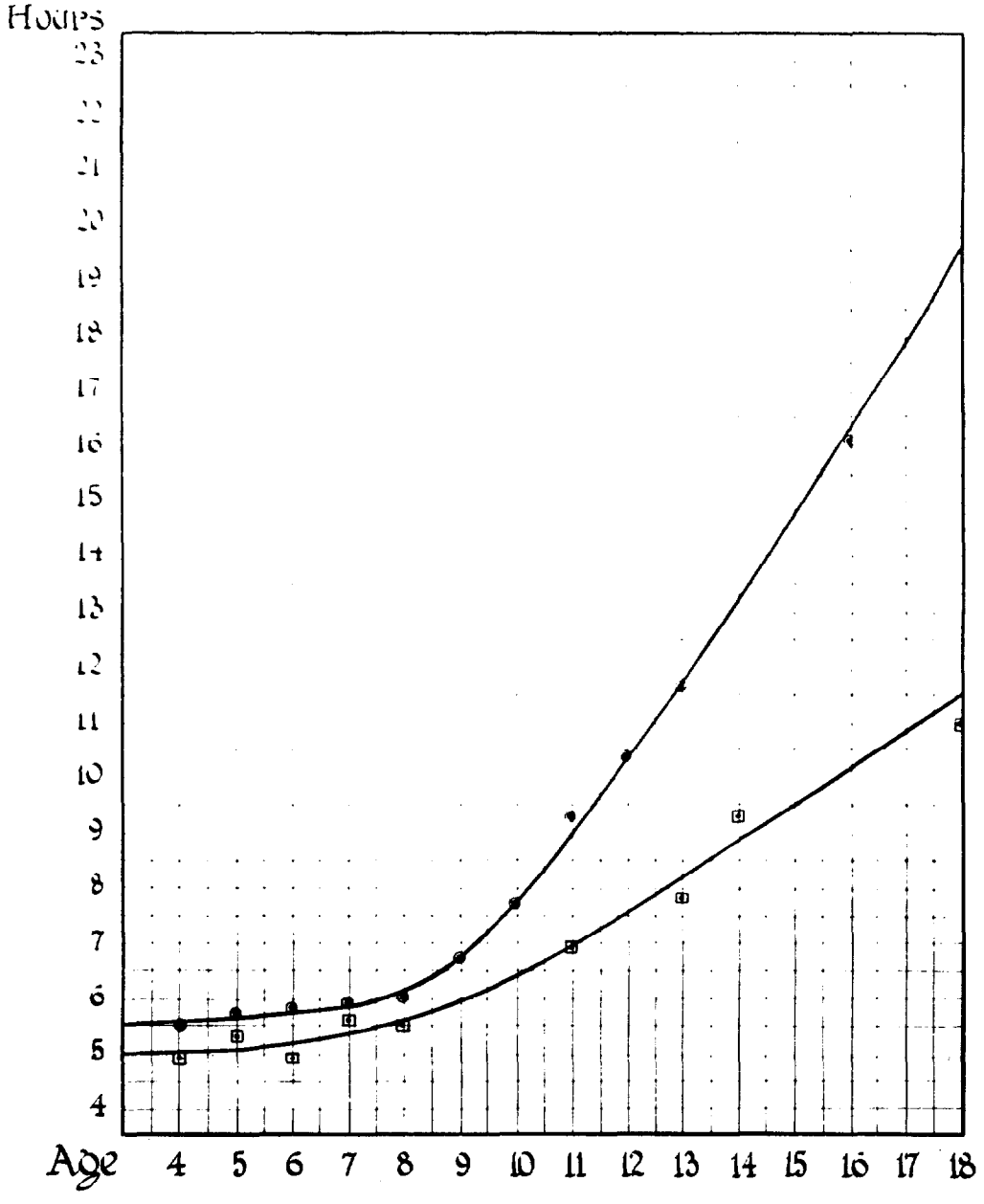

Chart 1. Showing the latent period of tissue cultures in terms of the incubation age of the embryo whence the fragments were derived. Media ● undiluted plasma; ⊡ plasma, diluted with equal parts of Rinzer's solution.
Abscissae represent incubation ages of the embryo. Ordinates are durations of latent period in hours.

patients we have been able to obtain curves of a portion of the left ventricular excursion which we have been able to compare with curves taken at a later time. We have studied the effect of digitalis in this manner in four patients and in these four there was a definite measurable increase in the extent of the excursion after an amount of digitalis had been given sufficient to produce a therapeutic effect and one showing characteristic changes in the T-waves in the electrocardiogram. This change often amounted to double the extent of the excursion previous to digitalis administration (Table 3). We believe that previous to these studies no direct evidence has been brought forward to show that digitalis in therapeutic doses has any effect in the human subject on the property of contraction. The result of this study is important for it is generally taught now that the action we describe does not take place. If the view we take is sound, digitalis should be reintroduced in the clinic for use in those cases in which heart failure is present and in which the mechanism is regular. Its disuse has been due to views based on experiments which laid emphasis on other functions than that of contraction.

Table 3.

Effect of Digitalis on the Left Ventricular Excursion Studied by means of X-Ray Curves.

Hosp. No.	Cardiac mechanism	Date	Digitalis	Rate per minute	Left ventricular excursion in mm.
4814	Normal rhythm	10-5-23	0	120	3.7
		10-20-23	2.1 gm. after digitalis	90	6.6
		11-7-23		85	2.9
4932 (1st series)	Auricular fibrillation	2-4-24	0	144	5.2
		2-6-24	1.5	108	10.9
(2nd series)		2-29-24	0	111	4.5
		3-7-24	1.5	92	11.7
4937	Normal rhythm	2-7-24	0	108	5.8
		2-8-24	0.9	105	8.6
		2-14-24		105	6.3
		2-18-24	1.2 after digitalis.	101	9.2
		2-28-24		105	6.9

We have treated a few patients showing cardiac edema with calcium chloride by mouth to test its efficacy as a diuretic. We have done this in the search for means for combating cardiac edema in patients who fail to respond to digitalis and to the usual diuretics, theocin and diuretin. From the few cases we have studied it was seen that calcium chloride given in doses of 15 gms. by mouth a day for 3 to 5 days actually produces diuresis, but the amount of increase in urinary output was never conspicuous nor was the effect increased when giving calcium chloride in combination with digitalis. This procedure has already been recommended. On the clinical side Singer and Starkenstein in Germany and Danielopolu and others in Rumania report striking diuretic effects of calcium chloride given intravenously, in cases which did not respond to digitalis given alone. These authors used from 0.1 gm. to 0.5 gm. calcium chloride intravenously a day with excellent results and when combined with digitalis Singer obtained a diuresis of 7000 to 8000 cc. per day.

In order to test the matter further, we also intend to inject calcium in patients already digitalized. But before doing so we are taking the precaution of making preliminary observations in dogs.

A basis for attempting this form of therapeutics is contained in Loewi's experiments in which he believes to have shown that the combined exhibition of calcium and of digitalis results in a sensitization of the heart muscle to calcium by means of digitalis. A similar relation was reported by Clark who showed in perfusion experiments in frogs that the systolic action of digitoxin is dependent on the presence of calcium,and that diminution of the quantity of calcium diminishes the systolic action of digitoxin, while an excess is without effect.

We have injected from .5 to 1.1 gm calcium chloride intravenously in dogs without the appearance in the electrocardiogram of ventricular premature

contractions (as an evidence of increased irritability of the heart muscle) and without ventricular standstill. We have not injected amounts beyond this because 1.1 gm. is probably in excess of the amount we would use in patients or in dogs.

In one dog we injected intravenously .5 gm. of calcium chloride and 30 per cent of the calculated lethal dose of digitalis (tincture used), (30 per cent of the calculated lethal dose was assumed to be the therapeutic dose from the work of previous investigators, Cohn and Levy, Jamieson, Robinson and Wilson) at the same time. About 40 minutes after the injection numerous premature ventricular contractions appeared in short runs. These were still present at the end of 1½ hours, but had disappeared the next day. One week later when the experiment was repeated this same dog gave a similar response. One week after this, 25 per cent of the calculated lethal dose of digitalis was given and the same amount of calcium chloride without causing an irregularity.

A second dog was given 25 per cent of the calculated lethal dose of digitalis intravenously and this amount of digitalis did not cause an irregularity. Two hours later 0.5 gm. calcium chloride was given intravenously and premature ventricular contractions failed to develop. A week later, when 30 per cent of the calculated lethal dose was injected followed by the same amount of calcium chloride a slight extrasystolic irregularity occurred. It appears then that about 30 per cent of the calculated lethal dose is critical when combined with calcium.

We have been carrying forward the work which we began last year on the production of cardiac hypertrophy and heart failure in animals with the view to learning the pathological changes brought about in the heart and the physiological changes in the circulation. Although these general problems have long been the subjects of investigation, little has been done which is applicable to the problem we elected to study. This necessitated adapted

ion of methods and development of techniques suitable for our purposes. We decided for our experiments to use artificial valve insufficiencies as the basic injury. Last year we reported the progress we made in the following preliminary directions: (1) We devised a cardioscope for the purpose and worked out the technique for its use; (2) we adapted a technique for obtaining satisfactory X-ray photographs of the dog hearts; (3) we studied the problem of obtaining satisfactory blood pressure records on dogs and decided to use the Kolls-Erlanger method as the most satisfactory one available; (4) and we worked out doses of diphtheria toxin with which it might be necessary to supplement the valve injury in order to produce cardiac heart failure. The next phase in this general problem was that of obtaining samples of mixed venous and of arterial blood for the study of the blood gases in these animals. The obvious method was to obtain the blood samples by direct punctures of the right and left ventricles. Although this procedure is possible, in blood samples so obtained, the needle often penetrates the septum and one can, therefore, not be certain of the origin of the blood sample obtained. We made certain of this by observations on the cadavers of dogs. This method was accordingly discarded and we decided to obtain the sample of blood of the left ventricle from the femoral artery. The mixed venous blood we took directly from the right ventricle by inserting a cannula through the right external jugular vein. In order to accomplish this, it was necessary to devise a suitable trochar and cannula and to develop a technique for obtaining samples of blood by the use of this instrument. At first it was necessary to operate under guidance of a fluroscope but we afterwards discovered a position of the dog which allowed the cannula to slide into the right ventricular cavity fairly easily. This operation is done under novocaine without causing the animal any discomfort, and is performed under strict aseptic sur-

gical technique. The accompanying table gives analyses of arterial and venous blood obtained by these methods.

Table 4.
Blood Gases on Normal Dogs.

Dog No.	Date	Sample	O_2 Content Vol.%	O_2 Capacity Vol.%	% Saturation	CO_2 Content Vol.%
101	3-5-24	Arterial [=L.Vent]	16.90		92.6	41.67
		Rt. Vent.	14.09	18.25	77.2	47.49
90	2-27-24	Arterial	18.31	19.70	92.9	42.86
		Rt. Vent.	15.69		79.4	46.24
124	2-21-24	Arterial	19.05	20.14	94.5	44.02*
		Rt. Vent.	14.80		73.9	44.51*
131	2-19-24	Arterial	21.42	22.81	93.9	42.10
		Rt. Vent.	16.66		73.0	43.30
129	2-14-24	Arterial	15.70	16.58	94.6	43.77
		Rt. Vent.	13.03		78.5	45.58
122	2-5-24	Arterial	20.93	22.86**	91.6	40.87
		Rt. Vent.	17.44	22.97**	75.9	44.39
127	1-24-24	Arterial	18.01	20.90**	86.2	47.96
		Rt. Vent.	15.16	20.97**	72.2	56.12
132	3-12-24	Arterial	19.28	21.28**	90.6	40.43
		Rt. Vent	14.26	20.97**	68.0	42.36

* These results were checked.
** Note how closely the O_2 capacities of the Arterial and Rt. Vent. samples agree.

After perfecting these methods we returned to operating on the heart valves of dogs. We have operated on 7 more dogs. In these dogs data on blood pressure, the size of the heart by X-ray photographs, the electrocardiogram, and blood gases were obtained before operation. In three of these

the cardioscope was used, and in 4, the valvulotome. Two of the dogs in which the cardioscope was used began to fibrillate when the cardioscope was inserted and died of acute heart failure before any valve lesion had been produced. The third dog died from heart failure ½ hour after completion of the operation. At autopsy it was found that the mitral valve had been cut extensively. We attributed these three failures in succession to the extremely poor kind of dog with which we were supplied. For operations as extensive as these, the animals should be selected with great care. Because at this time no animals were suitable for operation with the cardioscope we used the valvulotome, with this modification in technique usually employed in its use: the valvulotome, was inserted into the left auricular appendage (instead of through the carotid artery as previous experimentors had done) just as the cardioscope had been inserted, and this enabled a better control of the instrument and of the injury which one wished to produce. Of the 4 dogs subjected to this operation, 3 are living and well, 3, 2, and 1 weeks, respectively, after operation and have systolic murmurs as evidence of mitral insufficiency. The 4th dog died 24 hours after operation of acute heart failure with bloody fluid in both chest cavities. On examining the heart, cordae tendinae of both flaps of the mitral valve had been cut and too great an insufficiency of the valve had resulted.

We shall use the valvulotome in small dogs which are not suitable for the cardioscope, and the cardioscope in the larger animals.

The effect of rapid heart rate per se on the circulation and ventilation of the blood is still an unsettled question. In an attempt to study this relation we have planned a type of experiment which we hope will resemble more closely physiological conditions in the normal dog than previous experiments have done. So far we have carried out 2 preliminary experiments. We have sewn two stimulating electrodes into the right auricular appendage, and

after insulation with rubber tubing brought the wires outside the chest wall and closed up the chest. After the animal has recovered from the operation we can stimulate the auricle by faradic current and make the auricles fibrillate, or we can stimulate with galvanic current at any rate desired and make the heart beat rapidly at a regular rate. Under either of these conditions it is possible to study the oxygen content of the arterial and mixed venous blood. The first dog on which we operated recovered satisfactorily. One week after operation we attempted to stimulate the auricle by means of the electrodes that had been sewed to this structure but failed. We then operated on the dog to find out the cause of the failure and to attempt to remedy it, and found that one of the silver wires had broken in the rubber insulating tube. The attachments of the electrodes to the auricle had held securely. This second extensive operation so soon after the first was too severe a shock. The heart stopped beating during the manipulation incident to examining the relations brought about by the previous operation. A second dog was later subjected to the same operation, using a stouter silver wire for the electrodes. This animal died on the 2nd day after operation from a fulminating pneumonia involving in complete consolidation all the lobes of the lungs. At autopsy the electrodes were satisfactorily in place. We expect to go on with these experiments.

With Dr. Crawford and with the cooperation of Dr.McIntosh, patients with edema as the result of heart failure have been studied, as with Dr. Stewart, but in these patients the agent employed in treating them has been novasurol. These were likewise patients whom one cannot relieve of edema by rest or by administering digitalis.

Novasurol is a preparation containing 33.9 per cent of mercury. The mercury is contained in a complex molecule and it is administered as a 10 per cent solution in doses of 1 to 2 cc. intramuscularly. Mercury has at

an earlier period been employed as a diuretic in the form of calomel first by Jandrassic in Budapest. A marked diuresis was reported to have resulted from its use but the method was abandoned on account of the injuries to the kidneys which it produced. Novasurol was introduced as a mercural in the treatment of syphilis. The toxicological effects brought on by its use in this disease were slight compared to those due to other mercurial preparations. Of importance is the fact that no kidney damage is found except in cases in which the kidneys are known to have been damaged initially.

The action of this drug has so far been studied in 4 patients. They were all cases in which the use of digitalis failed. The effect of the drug on the following functions has been investigated: (1) the effect on urinary output, (2) the effect on the extent of edema, (3) the changes in the heart's action as the result of the treatment, (4) the changes in the output of urinary chlorides, urea and ammonia, (5) changes in the blood urea and chlorides, (6) changes in the chlorides of the edema fluid, (7) the remote effects on the kidneys as estimated by the appearance of albumen, red blood cells and casts, (8) the mechanism of action of the drug and (9) the evidences of toxicity.

The administration of the drug has been followed in these cases by marked benefit. In about 2 to 6 hours after the administration, diuresis commenced and was continuous for about 24 hours. After this time the amount of urine diminishes. Specimens of urine were collected every three hours for a period of 3 days. On the second day the drug was administered. Specimens of venous blood were obtained each day at the same time, the time being selected to correspond to that at which diuresis was anticipated to be present. Edema fluid was collected when it could be obtained at the same time as the blood specimens. The output of urine often increases from 200 to 300 cc. in 24 hours up to 3000 cc. In the cases which are most severe the onset of di-

uresis is delayed. Diuresis is followed by a corresponding loss of weight and definite improvement in the symptoms. The administration is not repeated until after four days. During the latter part of this period the edema increases slightly. By repeated administration it is possible completely to remove the edema. Two cases of cardiac disability have received marked benefit from the loss of water effected in this way. In one case there was little subcutaneous edema but visceral congestion. In both cases there was little doubt as to the effectiveness of the drug, not only in improving the function of elimination, but also in relieving very severe distress.

The action in increasing the excretion of chlorides in the urine was striking. Not only is the concentration of chloride excreted per day increased, but so also is the absolute amount. Urea and ammonia may be increased or slightly decreased. Their concentration in the urine is decreased. In the blood the chlorides showed a slight fall; urea maintained about its previous level. The chlorides in the edema fluid, as was to be expected, followed closely the changes in the blood.

The accompanying charts show graphically the changes in output, weight, and in the composition of body fluids which were produced in one of the patients (Charts 2 and 3).

So far no evidence of any deleterious action on the kidneys has been found. Two cases have been observed for a period of three months. There has been in each case a marked decrease in the amount of albumen in the urine and in none has an increase in the number of red blood cells or casts been found. An increase in blood urea as an indication of kidney damage has been absent.

In one case the administration of novasurol was followed by a rigor lasting for about 15 minutes followed by a rise in temperature. There was also vomiting. Recovery was complete next day; there were no untoward after effects. The administration of the drug, however, was not repeated. Edema was less

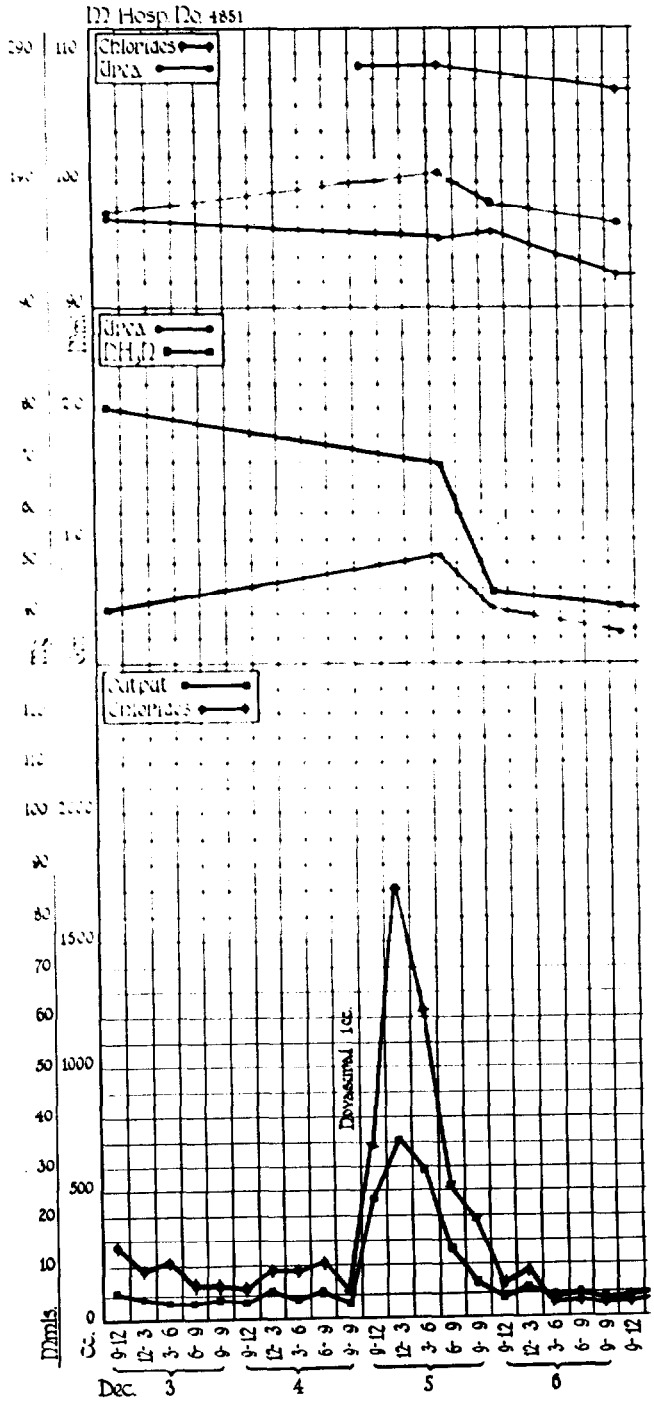

Chart 2.

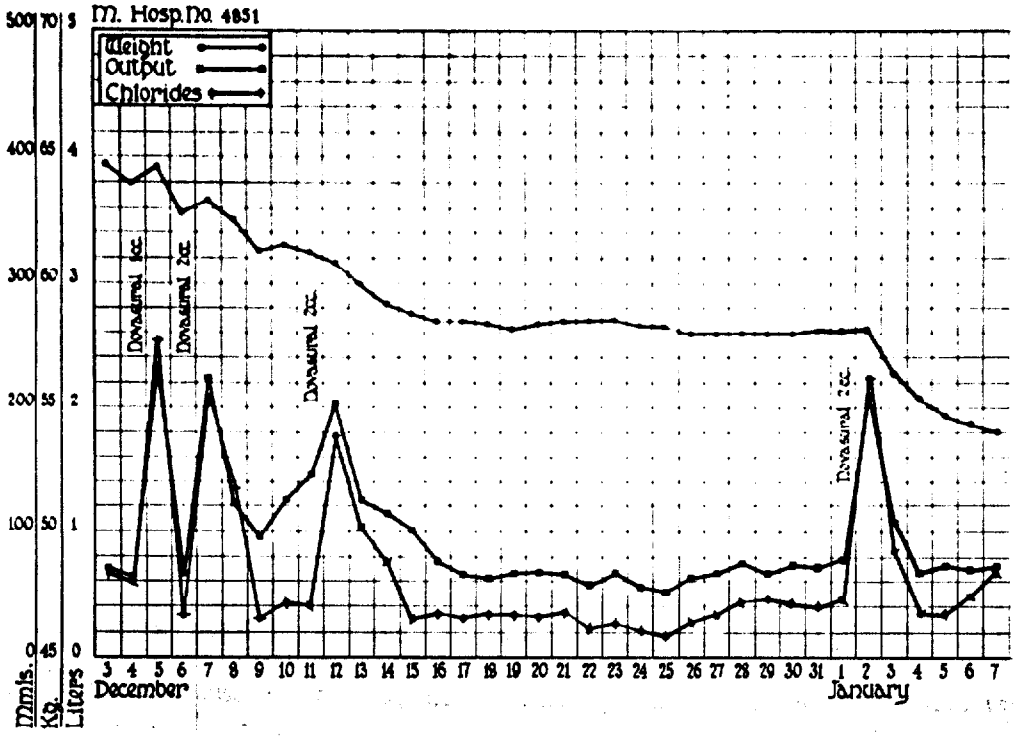
M. Hosp.No 4851
Weight
Output
Chlorides
Divasirol 4cc.
Divasirol 2cc.
Divasirol 2cc.
Divasirol 2cc.
500 70 5
400 65 4
300 60 3
200 55 2
100 50 1
0 45 0
Mmls. Kg. Liters
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7
December
January

Chart 3.

marked in this patient than in the others. Two other patients complained of slight headache on one occasion following the administration of the drug. Otherwise no toxic symptoms have developed.

Two conceptions for explaining the action of novasurol suggest themselves; first, by its action, fluid is removed from the tissues into the blood. In consequence the blood is diluted; as a result the excess fluid is excreted by the kidney. Second, fluid is in the first instance excreted by the kidney, the blood becomes concentrated and for this reason fluid is withdrawn from the tissues. In order to learn which of these assumptions is correct we have examined the hemoglobin percentage of the blood before, during, and after the diuresis and also have made hematocrit readings on the venous blood we obtained. In every observation we found a slight but definite increase in the hemoglobin percentage during the diuresis. The hematocrit readings also show a corresponding slight increase in the corpuscular content of the blood. In so far as these data may be regarded as evidence, they point to the probability that the action of the drug is exerted primarily on the kidneys.

We have estimated the excretion of phenolsulphonphthalein before, during, and after the onset of diuresis. The output of this substance was usually slightly decreased during diuresis. The amount increased progressively as the efficiency of the circulation improved following the removal of edema.

During the study of diuresis due to novasurol it was found that in the days following its administration a certain amount of the ground which had been gained was lost, that is to say, edema began again to increase. It was, as has been said, deemed inadvisable to inject the drug again before the expiration of four days. It is desirable to find another agent with which in the interval to remove the remaining fluid or at least to prevent its

increase. Diuretin was tried in one case without success. The use of theocin was not attempted. It occurred to us that urea might serve our purpose. Urea was accordingly given in doses of 30 gm. a day. With this, satisfactory results were obtained. In most cases there was an increase in the urinary output to about 1000 cc. a day. In most cases the extent of edema did not decrease but in no case did an increase occur, at least for a period of several weeks. When edema fluid tended to reaccumulate this was very gradual. Further studies are to be made to learn the effect of the administration of urea on diuresis.

During the administration of urea the changes in the constituents of the urine and blood were studied in the same manner as during the treatment with novasurol. In the urine it was found that the chloride excretion remained about the same as when the patient was receiving no medication. The urea in the blood was, of course, increased while the drug was being given as was also its excretion. When the drug was discontinued and time allowed for the quantity that had been given to be eliminated there was found to be no permanent rise in the level of blood urea. This method of treating cases of heart disease in which the urinary output remains consistently at a low level may have distinct usefulness. Its use has shown no evidence of a deleterious effect. In any case it appears not to have the disadvantage possessed by drugs of the class of diuretin and theocin.

The action of the sparteine sulphate on auricular fibrillation has been studied. From experiments formerly performed on dogs by Dr. Crawford there seemed to be good reason for the belief that the action of this drug was similar to that of quinidine sulphate. The drug has been given to four cases. In one case the normal cardiac mechanism was restored and has continued. In the other three the abnormal mechanism continued. Of the latter cases two were however, advanced cases in which success is relatively infrequent. In the

third case in which a favorable outcome might have been expected, quinidine also failed. Sparteine was administered in these cases in gradually increasing doses. Digitalis had previously been given to these patients. The first effect of the drug was an increase in pulse rate similar to that obtained after giving quinidine. This result differs from that reported in normal animals where a slowing of the heart rate is found. The drug was given until evidences of toxicity were obtained. The commonest of these was headache. Others were nausea. Abdominal discomfort and slight blurring of vision were recorded. These rapidly disappeared on stopping the drug.

Preliminary observations have been made of the methods in use for the study of capillary changes in disease. The questions in which we are particularly interested concern the role played by the capillaries in the circulation when the latter is failing. It is not only their morphology in various circulatory states which is of interest but also their mechanism. Do they exhibit the functions of contraction or that of peristalsis? What do they contribute to the dynamics of the circulation? In addition to these things it is desirable also to know the rate of the blood flow in them in conditions in which this presumably changes from the normal. What changes in their mechanism can be brought about by means of agents such as digitalis it is likewise important to study. It is of course possible to study the shape and size of the capillaries and the changes of pressure in them. But the technique which is available is insufficient for the detailed study required for investigating the questions which we have raised. It is, moreover, doubtful whether the ordinary methods of recording changes taking place in these structures such as drawing their outlines and simple photograpny, suffice for accurate and satisfactory study. The behavior of the flow of blood in the capillaries plays so fundamental a part not only in the dynamics of the circulation but also in the respiration and nutrition of the tissues, that it is

difficult to exaggerate the importance of an exact knowledge of it both in health and disease. We are especially eager, therefore, to avail ourselves of the opportunity of securing the services of Mr. Rosenberg,which have been placed at our disposal.

We have found in the first instance that in order to carry on satisfactory kinematography, improvements in technique are essential. The chief of these concerns the question of illumination. The oblique illumination which is customarily employed has disadvantages. We have, therefore, secured lenses, such as are used in petrology in order to test their usefulness in obtaining vertical illumination of the part to be studied. Enough has been said perhaps to show that even the technical problems connected with photography require further study before the proper study of the capillary circulation can be undertaken. It is important to urge, therefore, that for a year at all events the service of a technician of Mr. Rosenberg's ability be available, with the view at least to become assured of the usefulness of the kinematographic method for the studies we have in view.

Physical Chemistry of the Blood.

Dr. Van Slyke, Dr. Hastings, Dr. Salvesen, Dr. Linder, Dr. McIntosh, Dr. Cecil Murray and Miss Hiller.

Nephritis. The study of the metabolic cause of lipemia in nephritis by Dr. Linder and Miss Hiller has been concluded. After a meal of fat the blood fat in lipemic patients shows a greater rise above the fasting level than that of normal individuals or non-lipemic patients. There is evidently in the lipemic individual an abnormality in the mechanism which removes the fat from the blood. There are but two known modes of removal, viz. combustion and storage in tissue depots. The combustion was studied by following the gas metabolism after the fat meals. The increase in oxygen consumption and the fall in respiratory quotient were as rapid and great as in normal individuals. The fat burning mechanism, therefore, showed no detectible abnormality.

The cause of lipemia in nephritis is apparently attributable not to a loss of ability to burn fat, but to some disturbance in the process whereby it is deposited from the blood into the tissues.

Certain nephritics show high blood sugars. Dr. Linder, Dr. Salvesen and Miss Hiller are studying the metabolism and clinical conditions of such patients whenever they can be observed, in order to ascertain the nature of the disturbance in sugar metabolism and the connection between the hyperglycemia and the clinical type of nephritis. Some preliminary results indicate that a lowered pH may be an accompaniment and perhaps the cause of the hyperglycemia.

The metabolic behavior has been studied of calcium chloride, which has recently been considerably used as a diuretic in nephritis, especially by Blum of Strassbourg. It has been found, as was observed by Gamble in infants, that the calcium is excreted in the feces, and the HCl is absorbed. The result, we find, is a marked acidosis, both the alkaline reserve and the pH falling severely. Neither the blood calcium nor the urinary calcium excretion is affected. The diuretic effect is attributable to the HCl rather than to the calcium. The severity of the acidosis that may result from the dosage recommended is such as to contraindicate the treatment, at least unless the acid-base balance of the patient is accurately controlled.

Dr. McIntosh has collaborated with Dr. Crawford in studying the effect of kidney function of the arsenical, "novasurol," which has a remarkable diuretic action in edematous heart patients. It developed that while water excretion might be increased ten-fold and chloride excretion a hundred-fold, urea excretion is practically unaffected, except for the relatively small acceleration that Austin, Stillman and Van Slyke found to occur in normal individuals when the urine volume is increased. The peculiarity of this substance in stimulating the excretion of salt and water, but not of urea, in-

dicates the sharp differentiation in the mechanism by which the respective substances are excreted, and may be of assistance in physiological experiments on these functions.

Physical Chemistry of the Blood.

The study of the electrolyte and water distribution between the cells and plasma and between plasma and edema fluid, is being continued.

On the distribution between cells and plasma the effect of CO_2 changes was determined in the experiments performed last year in Peking. The results, as reported previously, accord with those predicted from the alkali-binding power of the blood proteins, from calculations based on Donnan's theory and the assumption that the ratio $\frac{\text{ions + molecules}}{\text{water}}$ is maintained equal in the cells and serum. The results of oxygen tension changes were calculated at the time, but were not determined. Experiments are now being begun to determine the oxygen effect. As the effect is less than that of CO_2 a more accurate chloride method for the cells was required, and was devised as outlined below. The preliminary experiments indicate that the effect of oxygen exchange on the chloride and bicarbonate distribution approximate that calculated from the Donnan theory and the difference in base-finding power between oxygenated and reduced hemoglobin.

The distribution of Cl', HCO_3', Na^+ K^+ and H^+ between blood plasma and edema fluid has been determined in a number of patients by Dr. Hastings and Dr. Salvesen. According to Donnan's theory of electrolyte distribution previously discussed, if the membranes separating serum and fluid are permeable to these ions, they should be so related in their concentrations that the relationship is

$$\frac{Cl_s}{Cl'_f} = \frac{HCO_{3s}'}{HCO_{3f}} = \frac{Na^+f}{NA+_s} = \frac{K^+f}{K^+s} = \frac{H^+f}{H+_s} = r$$

where sub_s indicates serum and sub_f edema fluid. This equality of the ratios

was found to hold for all the ions except potassium. There is some doubt concerning the accuracy of the method used for K in serum, and it will be investigated. For the other ions there was observed not only the approximate equality of the ratios but also a value of r almost exactly that calculated from the difference in alkali binding power between proteins per liter of serum and those of the edema fluid.

The study of the chemical basis for the physiologically important fact, that reduced blood absorbs more CO_2 at the same tension than does oxygenated blood, has been continued with Dr. Hastings and Dr. Murray. It was shown last year that the cause of the phenomenon is that oxygenated hemoglobin binds more alkali than reduced, at physiological pH. It has now been found by more accurate and detailed experiments that the difference in base-binding power between the two forms of hemoglobin varies with the pH, being at a maximum at pH 7.4 and decreasing in a regular curve on each side of this point. Quantitatively the curve is that calculated on the assumption that a single acid group in the hemoglobin molecule has its acidity as measured by its dissociation constant, increased about 25 fold by the change from reduced to oxygenated hemoglobin.

The degree of ionization of the sodium, potassium and calcium salts of the serum proteins and of hemoglobin is being studied by Dr. Hastings and Dr. Cecil Murray with preparations of crystalline hemoglobin made by Heidelberger's method, and of electrolyte-free serum albumin and globulin made by Miss Hiller. The determinations have been made by the electrometric method with amalgams of the alkalies studied as electrodes. With a technique obtained in part from Professor Harned of Philadelphia, Dr. Hastings has obtained consistent results with the sodium and potassium salts indicating that they are about 60 per cent ionized. This ionization about equals that of sodium bicarbonate

The determination of the state of the blood calcium offers problems peculiar both in difficulty and in physiological and clinical interest, as is evidenced by Salvesen's work outlined in our last report. Hastings and Murray are endeavoring to determine the ionization of calcium-protein salts, and also the nature of the factors which enable the blood to hold in solution much more calcium than a simple water solution containing bicarbonate, phosphate and pH equal to those found in the blood serum.

The study of the physiology and pathology of the acid-base balance of the blood begun in connection with the diabetic clinic in the hospital was last year extended to pneumonia. Drs. Hastings, Morgan and Neill showed that the departure from the normal acid-base balance is slight and is in the direction not of acidosis, as previously assumed, but of an alkalosis, due to driving off CO_2 by the rapid ventilation. These results during the febrile state of a somewhat lowered CO_2 tension in the blood, an increased pH with unchanged alkaline reserve.

The results, together with other facts which have been uncovered in this and other laboratories, have led us to take up again the study of the mechanism controlling the acid-base balance and connecting it with the respiration. The preliminary results may be summarized as follows: When the alkali reserve of the blood is altered (as in diabetic acidosis or in the opposite direction, as in loss of HCl from pyloric stenosis and vomiting) even slight alterations are accompanied by pH changes. The earlier conception that, teleologically expressed, the pH is so important that the organism will alter the CO_2 tension to the respiratorily possible limit in order to prevent the slightest change in pH, was based on data in the literature which were incomplete, and in some points inaccurate. It appears, on the contrary, that when the alkali reserve is lowered, the percentage change in the hydrion concentration

is usually about twice as great as that in the CO_2 tension, and this ratio is maintained to the extreme limit of acidosis. Judging from the compromise between change in H^+ concentration and in CO_2 tensions to which the organism gravitates when the blood alkali is altered, normality of CO_2 tension is about twice as important as normality of H^+ concentration. And, because of this compromise, even a moderate acidosis in the sense of a lowered alkali reserve is, as a rule, also an acidosis in the sense of a lowered pH, an "uncompensated acidosis." Experimental work on this problem is being done by Dr. Hastings and Dr. Murray, and promises to explain some of the confusion that has existed among physiologists concerning the relative importance of CO_2 tension and pH in controlling the respiration.

Methods of Blood Analysis.

Blood Gases. The technique for use of the constant volume apparatus has been developed in such a manner that the same apparatus used for analysis of the usual amounts, 1 or 2 cc. of blood, may also be used for both O_2 and CO_2 determinations on as little as 0.2 cc. of blood, with an accuracy approaching 1 per cent. As absorbent for oxygen, sodium hydrosulfite ($Na_2 S_2 O_4$) has been introduced in place of pyrogallol. The technique for carbon monoxide determination with the new apparatus has also been worked out, so that this gas can be determined as easily and about as accurately as O_2 and CO_2. The three gases, CO_2, O_2 and CO can be accurately determined on a single 1 cc. sample of blood in about 20 minutes. For this purpose the gases are freed by addition of lactic acid and potassium ferricyanide. The CO_2 is absorbed by a few drops of KaOh and the O_2 is absorbed with 0.5 cc. of hydrosulfite solution, the pressure of the gases being measured on the manometer before and after each absorption. The residual gases are CO and N_2, which are measured together, the CO being estimated by subtracting 1.15 volume per cent for the N_2, which is constant figure for the

circulating blood. Technique has also been ascertained for measuring the CO by absorption with a cuprous chloride solution, but the N_2 content of blood is so constant that estimation of the CO by subtracting 1.15 from the CO + N_2 per cent proves as accurate as the absorption.

Blood Chlorides. In measuring the cell chlorides with the accuracy required to follow the changes observed in our experiments on electrolyte distribution, it was found desirable to obtain a technique which obviated precipitation of the blood proteins, and the errors due to the volume of precipitate, especially bulky in the case of separated cells. The problem was solved very simply by digesting the blood or cells with 3 volumes of concentrated nitric acid containing a known amount of silver nitrate. The test tube or flask containing the mixture was allowed to stand immersed in steam or boiling water until the fluid became a clear light yellow, one hour sufficing for serum, several hours being required for whole blood. The excess silver was titrated with sulfocyanate in the same vessel so that the entire analysis was performed in a single container. The method has obviated the difficulty of bulky hemoglobin precipitates, at which it was aimed, but it has also proven so simple that it is being used in preference to previous procedures for routine plasma analysis. The accuracy of the method was tested by precise analyses of standard chloride solutions, and of control solutions made by adding to dialyzed blood known amounts of chloride. The experimental work was done by Mr. Julius Sendroy, at present technician in the laboratory.

Dr. Salvesen has continued, with Dr. McIntosh and Dr. Hastings, his study of the physiological phenomena connected with changes in the calcium content of the blood serum. As stated in a former report, his studies of the mineral and protein constituents of the blood plasma in nephritics led to the conclusion that the fraction of calcium bound to protein could be dec-

reased without causing tetany, which results only from a loss of the diffusible, and presumably ionized Ca. That loss of diffusible Ca is itself a primary cause of tetany has been somewhat disputed, increased Na or pH being suggested as the important factors. Dr. Salvesen is now able to produce tetany at will in dogs in a few hours merely by oral administration of several grams of neutral or alkaline sodium phosphate. The Na and pH of the serum are unchanged. The serum PO_4 is about doubled, and the Ca reduced to about half the normal value. That the tetany is due to the Ca loss rather than PO_4 increase is shown by the fact that it is instantly cured by intravenous injection of Ca Cl_2 sufficient to restore a normal blood Ca content, the PO_4 remaining unchanged.

Acute Respiratory Diseases.

The number of cases of acute respiratory diseases applying to the hospital for admission during the winter has been less than during previous years. This fact is directly related to the fact that the number of cases of acute respiratory disease in New York City have undergone a very marked diminution this year in comparison with previous years. While the opportunity for clinical study has, therefore, not been so great, the study of the more fundamental problems relating to pneumonia has proceeded satisfactorily. Attention is directed to the results of the work of Dr. Avery relating to the study of ferment and other metabolic activities of the pneumococcus and especially to the results so far obtained by Dr. Heidelberger and Dr. Avery in determining the chemical nature of those substances produced by pneumococcus upon which type-specificity depend. The work has reached a stage in which the conclusion seems justified that these specific substances have the chemical structure of polysaccharides. Moreover, the very important discovery has been made that the soluble specific substance produced by Type III

pneumococcus differs chemically from that produced by Type II pneumococcus. The study of these substances is being continued and, in addition, work is being undertaken to determine the chemical nature of the soluble substance produced by Type I pneumococcus.

Other studies relating to pathogenesis of pneumonia and immunity to pneumococcus infection have been carried on by Dr. Stillman and Dr. Branch, Dr. Reimann, and by Dr. Sia of Pekin Union Medical College who has acted as a voluntary assistant. Studies relating to the character of the abnormalities in the respiratory function occurring in pneumonia have been continued by Dr. Binger and Dr. Brow. The oxygen chamber has been completed and has been successfully used, though, so far, only a small number of cases have been treated in the chamber. The experimental studies by Dr. Binger and Dr. Brow have led to important conclusions, namely that the rapid shallow breathing of pneumonia is probably primarily related to nervous stimuli arising in the infected lung, rather than to changes in the environment of the respiratory center. The work also indicates that the pathological changes in the blood vessels of the lung may be directly related to the abnormalities in the nervous respiratory mechanism. These observations are not only of academic interest but may be of considerable significance in the therapy of lung infections. The more detailed reports of the studies on acute respiratory diseases follow.

<u>Studies on Biology of Pneumococcus</u>.

Dr. O. T. Avery, Dr. H. J. Morgan, Dr. J. M. Neill.

A study of the biology of pneumococcus, which has been continued in the bacteriological laboratory of the hospital during the past six months, has yielded certain facts which are not only of interest with reference to the physiology and chemistry of the bacterial cell but which give promise of wider significance in the interpretation of the processes of infection in

the animal body. This investigation as conducted at present follows two main lines of development. One of these is concerned with reactions of oxidation and reduction which are exhibited both by the living cell and by sterile extracts of the active intracellular substances upon which these processes depend. The other line of development relates to the immuno-chemistry of the cell constituents of pneumococcus. The progress made thus far in this study has already revealed the interesting fact that definite relationships exist between the chemical constitution and the biological specificity of these cellular substances. These observations, recorded in more detail in another part of this report, furnish a basis for the better understanding of the serological and antigenic properties of pneumococcus and hence are specifically related to the more general problems of pneumococcus infection and immunity.

1. Oxidation and Reduction by Pneumococcus. The study of certain oxidative reactions of the bacterial cell has been stimulated by the earlier observations on the influence of certain catalytic agents upon bacterial growth. Plant tissue in its natural unheated state has been found to possess certain accessory substances which greatly favor the growth of bacteria. Among these substances the vegetable oxidases have been found of considerable importance. Where the oxidation and reduction system of vegetable tissue has been preserved this tissue has been found capable of replacing blood in the cultivation of the so-called hemophylic organisms; of greatly accelerating the growth of pneumococcus and other Gram-positive cocci, and of making possible the aerobic growth of anaerobic bacilli. The plant oxidases together with other accessory substances in the tissue seem to meet certain physiological needs of the bacterial cell not wholly provided for by the ordinary culture media. Moreover, there are bacteria which are either devoid of or incompletely provided with an efficient oxidase system. It has recently been

shown that bacteria which possess no demonstrable catalase form peroxide whenever the cells are grown with free access to air. Pneumococcus has apparently no catalase and little or no peroxidase. Whenever this organism is grown in media exposed to air, peroxide accumulates in the culture fluid. This peroxide having the properties of hydrogen peroxide is toxic and even bacteriacidal. When plant tissue is present in the medium, however, there is artificially supplied an active oxidizing mechanism which functions not only in destroying these deleterious products but which may also serve to furnish energy for the initiation and maintenance of growth. Study of the cultural conditions which favor the formation and accumulation of peroxide in the medium has shown that the peroxide-forming activity of pneumococcus is a function not dependent upon the presence of living cells. By special procedures, sterile extracts of pneumococcus free from all living and formed cells have been found to contain substances which are reactive with molecular oxygen. Among the oxidation-reduction activities of these extracts already reported are the prompt formation of peroxide when the extracts are exposed to air, the consumption of molecular oxygen, and the active reduction of methylene blue.

The peroxide which accumulates as a result of this oxidative process has in turn been found to cause the destruction of other active intracellular agents, sich as pneumococcus hemotoxin. If the oxidation is allowed to proceed in the presence of oxyhemoglobin, this substance is rapidly converted to methemoglobin. This phenomenon affords an explanation of the mechanism by means of which these blood changes are brought about by the living cell. More recently Dr. Neill has shown that certain enzymes of pneumococcus are also destroyed by the oxidizing agents which are formed when sterile extracts of the cellular substances are exposed to air. The carbohydrate hydrolyzing enzymes (sucrase,

raffinase, inulinase, amylase) proved most easily inactivated while pneumococcus lypase, peptonase, were unaffected by oxidation. This study brought proof that a number of hydrolyzing enzymes of pneumococcus are destroyed by oxidizing agents actually formed by constituents of the same cell from which the enzymes are derived.

The Influence of Artificial Peroxidase Upon the Growth of Anaerobic Bacilli. Since sterile unheated plant tissue was found to facilitate aerobic growth of a number of anaerobic bacilli it seemed of interest to determine whether or not it was possible to associate this function with some simpler inorganic tissue constituent. Since iron is known to exert an accelerating action upon certain cellular oxidations, and is commonly found in conjunction with the peroxidase of plant tissue, it seemed possible that this substance might function in the oxidative mechanism of the bacterial cell and in the destruction of toxic peroxides in a manner analogous to that of plant tissue. As ferrous sulfate is known to accelerate many oxidation and reduction processes, and exhibits the usual reactions of peroxidase, iron in this form was chosen for study. However, when a solution of ferrous sulfate is added to broth, precipitation occurs. To overcome this, use was made of the method employed by Dony-Hennault in the preparation of artificial laccase. A solution of gum arabic and ferrous sulfate was precipitated in alcohol. The resulting precipitate is soluble in water and in aqueous solution gives the reaction of peroxidase with benzidine and hydrogen peroxide. Solutions of the gum-iron preparation remain stable in bouillon, the gum apparently functioning as a protective colloid. Quantitative analysis of this preparation shows that it contains approximately 20 mg. of iron per gram. In broth containing small amounts of this preparation the obligate anaerobes studied were found to grow through repeated transfers in the presence of air.

The fact that an inorganic salt of iron can by itself replace plant tissue in the aerobic growth of anaerobic bacilli lends support to the hypothesis previously advanced. It has been shown that certain aerobic organisms which are devoid of catalase form hydrogen peroxide when grown in the presence of air. In the case of pneumococcus which possesses no catalase, hydrogen peroxide is known to accumulate in the fluid of aerobic cultures in concentrations which are bacteriostatic and even bacteriocidal. As far as is known anaerobic bacteria are also devoid of catalase and hence these cells cannot destroy peroxides. From these relations it seems not unlikely that anaerobic bacilli 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 action of peroxides which may be formed as the result of the union of molecular oxygen with some autoxidizable substance in the bacterial cell. Under these circumstances organisms which are peculiarly sensitive to the action of these peroxides not only fail to grow but actually die. If this assumption is correct, then the aerobic growth of obligate anaerobes both in the presence of artificial peroxidase and of plant tissue finds partial explanation at least in the fact that peroxides formed are rapidly broken up under these cultural conditions. Therefore, so far as the toxic action of peroxides is concerned, the sensitive cell is protected almost as effectually as though it were growing under anaerobic conditions.

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1. Studies on Oxidation and Reduction by Pneumococcus.
III. Reduction of methylene blue by sterile extracts of pneumococcus.
O. T. Avery and J. M. Neill.

2. Studies on Oxidation and Reduction by Pneumococcus.
IV. The destruction of hemotoxin by sterile extracts of pneumococcus.
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V. The destruction of oxyhemoglobin by sterile extracts of pneumococcus.
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4. Studies on Oxidation and Reduction by Pneumococcus.
VI. The oxidation of enzymes by sterile extracts of pneumococcus.
J. M. Neill and O. T. Avery.

5. Methemoglobin Formation by Sterile Culture Filtrates of Pneumococcus.
H. J. Morgan and J. M. Neill.

6. Correlation of Certain Growth Phenomena Occurring during Growth of Pneumococcus.
H. J. Morgan.

7. A Case of Relapsing Trypanosomiasis Treated by Tryparsamide.
H. J. Morgan.

Studies on Immuno-Chemistry of Pneumococcus.

Dr. M. Heidelberger and Dr. O. T. Avery.

The so-called "soluble specific substance" of the pneumococcus, it

from pneumococcus cultures, and was found to be present not only in the intact bacterial cell as well, but also in the body fluids of the infected host. It was selected as a basis for the present studies on the chemistry of bacterial specificity because it was not only highly type-specific, but also possessed a stability to heat, enzymes and many chemical reagents that augured well for its susceptibility to study by the methods of organic chemistry.

At the time of the last report the soluble specific substance of the Type II pneumococcus had been purified to form a faintly yellow amorphous substance with a specific optical rotation of +55.2°, and nitrogen, carbon and hydrogen contents of 0.46, 46.3, and 6.0 per cent, respectively. It yielded 70.5 per cent of reducing sugars on hydrolysis and reacted with homologous immune serum at a dilution of 1:10,000,000 (Prepn.21 in Table I).

Table I.

Soluble Specific Substance of Pneumococcus Type II.

Prep. No.	Specific Rotation	Nitrogen	Reducing Sugars on Hydrolysis	Carbon	Hydrogen	Pptn with Immune Serum
		o/o	o/o	o/o	o/o	1:
21	+55.2°	0.46	70.5	46.3	6.0	10,000,000
21 A	+55.8°	0.20	67.2			5,000,000
22 A	+52.2°	0.41	65.8			5,000,000
22 B	+52.5°	0.31	62.6			5,000,000
23	+53.9°	0.39	62.3			5,000,000
24	+58.2°	0.16	74.8			2,000,000
25	+63.2°	0.18	80.3			3,000,000

From 21 on, Type II S.S. in 1:200 soln. gives no pppt. w. $CuSO_4$, no biuret reaction, no ppts. with phosphotungstic acid, tannic acid or neutral Pb. acetate, but ppts. w.H, basic Pb. acetate and $UO_2(NO_3)_2$. No color with iodine.

Soluble Specific Substance of Pneumococcus Type III.

27	-33.0°	0.11	73.0	42.3	5.2	2,000,000
28	-34.0°	0.05	73.0	42.6	5.6	3,000,000

Type III S.S. in 1:200 soln. ppts. with $CuSO_4$ and other heavy metal salts and gives no biuret reaction or ppt. with tannic acid. Phosphotungstic acid gives a turbidity perhaps due to the free Type III acid which is sparingly soluble and is pptd. by strong HCl. No color with iodine.

Subsequent work on the Type II specific substance has been devoted to improvement of the method of purification and attempts to separate the specific function from the polysaccharide portion of the product.

The method of purification previously described has been improved by the following additional steps: the active material is precipitated twice from alkaline solution by means of alcohol, and is then thrown out of solution three times with solid ammonium sulfate instead of twice. The final dialyzed solution is now concentrated to small bulk and poured into 10 -15 volumes of redistilled acetone, precipitating the soluble substance as a white, friable, fibrous mass. In this way preparations such as 21 A, 24 and 25 were prepared, with nitrogen contents of 0.2, 0.16 and 0.13 per cent, respectively. In the case of Preparation 25 the yield from 307.5 liters of culture fluid was 4.5 g.

In Preparation 23 both the source of material and the method of purification were varied, but essentially the same type of product was obtained. Unwashed pneumococci were dissolved with the aid of bile, the bacterial neucleoprotein was precipitated from the solution by acidification with acetic acid, and the soluble specific substance removed from the filtrate by adsorption on aluminum hydroxide. The adsorbate was washed with water and the specific substance recovered by extraction with disodium phosphate solution. By repeated fractionation of the resulting solution with the acid of alcohol much as in the usual method, bile substances were eliminated and a highly active specific substance obtained which resembled in its general properties the products obtained directly from the culture fluids (See Table I).

As it seemed possible that the polysaccharide found in the above preparations might be a tenaciously adheringimpurity, and that the actual specific substance might belong to some other class of organic substances, repeated efforts have been made to effect a separation and are still in progress. These experiments are along three main lines:

1. Hydrolysis by means on Enzymes. The common polysaccharide-cleaving enzymes, such as invertase, malt, diastase and pancreatic and salivary amylases, fail to produce reducing sugars in the solutions of the specific substance and leave the specific reaction unaffected as well. It has likewise been impossible thus far to detect any alteration of either the specific or carbohydrate function by the action of any molds grown in solutions of the active material. Experiments with molds, yeasts and bacteria will be continued.

2. Precipitation with Immune Serum. A solution containing 0.3 g. of Preparation 24 was precipitated with Type II immune serum, of which 800 cc. were required. The precipitate was washed with salt solution and coagulated by boiling it with very dilute acetic acid. The coagulum retained the specific substance, but this was finally removed, together with much protein material, by repeated extraction on the water bath with normal ammonium hydroxide solution. From the concentrated extract it was possible by a number of fractionations with alcohol, to recover 0.1 g. of a product with the properties of 24 A. (See Table II).

Table II.

		Specific Rotation	Nitrogen	Reds. Sugars on Hydrolysis	Pptn with Immune Serum
Combined and pptd. with $UO_2(NO_3)_2$	22A	+52.2°	0.41	65.8	1: 5,000,000
	22B	+58.5°	0.31	62.6	5,000,000
Recovered	22F	+53.9°	0.12	72.5	2,000,000
	24	+58.2°	0.16	74.8	2,000,000
24 Pptd. by immune serum; recovered	24A	+50.0°	1.0	74.3	2,000,000
24 Pptd. by basic Pb. acetate; recovered	24C	+50.0°	0.19	75.0	5,000,000

This preparation, in contradistinction to purer samples of the specific substance, gave a weak biuret reaction and a slight haze with tannic acid, indicating that the higher nitrogen content was due to contamination with protein decomposition products derived from the serum. The fact that the activity with immune serum was at least no higher, also justifies this interpretation. It will be seen, therefore, that after the precipitation of purified soluble specific substance by its own antibody, the subsequent dissociation of the immune precipitate resulted in the recovery of a polysaccharide which was practically identical with the original material.

3. Precipitation with Inorganic Salts. The only heavy metal salts thus far found which precipitate the Type II specific substance are the uranyl salts, which do not precipitate ordinary polysaccharides, and basic lead acetate, which precipitates all sugar derivatives. It will be seen from Table II that the material recovered after precipitation with these reagents was still a polysaccharide derivative practically nitrogen free and essentially unchanged in optical rotation, in activity with immune serum, and in percentage of reducing sugars on hydrolysis.

The following experiment is appended as showing not only the remarkable stability of the specific substance to strong acid in the cold but also in a rough qualitative way, how the specific reaction diminishes only as the polysaccharide is hydrolyzed by strong acid and reducing sugars appear:

1:1 hydrochloric acid used at room temperature.

Original concentration of Prepn. 21 : 1 : 1000.

Test No.	0	1	2	3	4	5
Time	0	2 hrs.	19 hrs.	2 days	3 days	6 days
Immune Serum	+++	+++	+++	+++ −	+	−
Cu. Red'n.	−	−	−	+	++	+++

Regarding the reducing sugar which is formed on hydrolysis of the Type II specific substance in its present state of purification, it was shown in the last report that glucosazone was obtained from the reaction mixture. This establishes glucose, fructose or mannose as the chief possible reducing sugars present. By following the optical rotation of a preparation during the hydrolysis, however, it was found that the initial high detrorotatory value remained essentially unchanged, whereas if mannose or the levorotatory fructose had been formed in appreciable amount, the rotation would have decreased as the hydrolysis progressed. It thus appears that glucose itself is the chief unit from which the polysaccharide structure is built up.

Summarizing, then, the work to date on the Type II specific substance it appears that, in its present state of purity the material is a white, amorphous sulphur and phosphorous-free polyglucose derivative in which the non-carbohydrate portion of the molecule represents 25 - 30 per cent of the total; that the low nitrogen content, 0.1 - 0.2 per cent and absence of reactions for protein split-products exclude relationship with the group of proteins and their derivatives, and that by all the methods hitherto used for purification, including adsorption as well as precipitation with the specific antibody, essentially the same type of polysaccharide derivative is recovered. It is, therefore, becoming increasingly difficult to believe that the carbohydrate present can be merely a tenaciously adhering impurity.

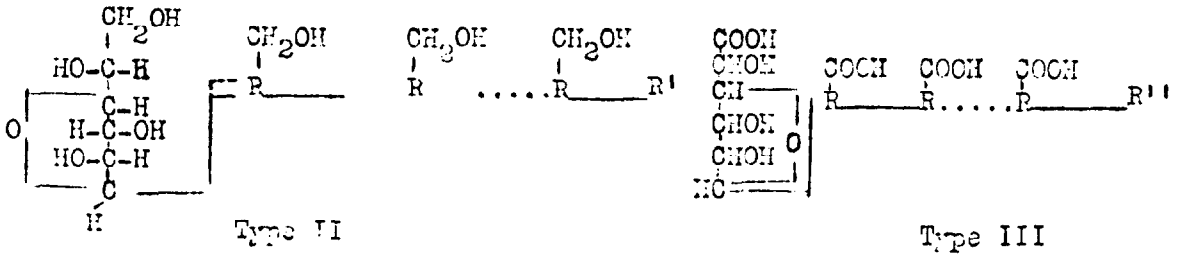
Type III.

Little work was necessary on the specific soluble substance of Type III pneumococcus to show that marked chemical differences existed between it and the corresponding substance of Type II.

The method used for the purification of the Type III specific sub-

stance is the same, up to the final stage, as that used for Type II. 8-day autolyzed broth cultures are concentrated to one-fifteenth volume on the water-bath and precipitated with 1.3 volumes of alcohol. High-spped centrifugation of the precipitate results in the usual 3-layer separation of which the middle, solid layer contains practically all of the active material. The remaining precipitations with alcohol and ammonium sulfate differed from those in the case of Type II only in revealing differences in the tenacity and appearance of the precipitates of active material.

It then developed that not only is the Type III specific substance precipitated by heavy metal salts such as those of silver, mercury and copper, which do not precipitate the Type II substance, but that, in conformity with this, the Type III substance is the soluble alkali or alkaline-earth salt of an insoluble, strong acid, which is thrown out of solution when an excess of strong hydrochloric acid is added to concentrated solutions of the Type III substance. Preparations 27 and 28 were purified by two such precipitations of the insoluble acid from strong solutions of material which had been carried through the usual method of fractionation. It will be seen that when redissolved with the aid of a little alkali the solution is levorotatory instead of dextrorotatory as in the case of the Type II substance; that the yield of reducing sugars and the degree of activity with immune serum are about the same; that the amount of nitrogen is negligible, and that the active substance again appears to be a polysaccharide derivative. The percentages of carbon and hydrogen are 4 and 0.6 per cent, respectively, less than in the case of the Type II substance, in accordance with the conception that a number of terminal CH_2OH groups in the polysaccharide molecule have been oxidized to COOH, thus giving rise to a strong acid. Provisionally the differences between the two types might be expressed by the rough tentative structural formulas.



Type II

Type III

in which $-\overset{CH_2OH}{\underset{|}{R}}-$ in one case is a repetition of the glucose unit on the left and $-\overset{COOH}{\underset{|}{R}}-$ in the other the radical of an acid such as glucuronic acid, in which the terminal $-CH_2OH$ group of glucose is oxidized to $-COOH$. The sugar units may be combined either in glucosidic union, or by polymerization, and other sugar units may be present in either molecule. R' and R'' represent the remaining 25-30 per cent of the molecule to which no clue exists as yet.

That some such difference actually obtains is indicated by the study of the hydrolysis products of the Type III substance, as yet only in its preliminary stage. With phenylhydrazine a crystalline osazone is formed. This softens above 180° and melts with decomposition at about 200°, but shows entirely different solubilities from those of glucosazone, nor does it mutarotate appreciably in pyridine-alcohol. p-Bromphenylhydrazine also yields a crystalline osazone melting at about 190°, with an initial specific rotation of about -280° and a final value of -230°. Definite data on the osazones of glucuronic acid and its analogs do not exist except that the p.bromphenyl osazone of glucuronic acid has a very high negative rotation.

To summarize, then, the soluble specific substance of Type III differs chemically from that of Type II in being an optically levorotatory strong acid, hydrolyzing to reducing sugars, chief of which is perhaps glucuronic acid or an analog, and not glucose, as in Type II.

pathogenesis of Lung Injections.

Dr. Stillman and Dr. Branch.

The study of the experimental production of acute lobar pneumonia in mice by the inhalation method has been continued. Normal mice have been intoxicated with alcohol and then exposed to air containing pneumococci in suspension, but in no case has a true pneumonia resulted. If mice are partially immunized, however, by exposure to an atmosphere containing live or even dead pneumococci in suspension, and they are subsequently intoxicated and again sprayed with live pneumococci, the lungs may show definite gross consolidation of one or more lobes. Sections of these lungs exhibit pathological changes resembling red and grey hepatization.

In order to see if a generalized immunity may be developed following inhalation of pneumococci, mice were sprayed from 2 to 12 times with pneumococci. The immunity of these mice was then tested by intraperitoneal injection of pneumococci. The results show that a definite general immunity against pneumococci may be developed in mice following repeated spraying with live organisms. No general immunity, however, has been found in mice which have repeatedly been sprayed with killed culture, or with pneumococcus vaccine.

Effect of Environmental Conditions on the Serological Properties of Pneumococcus

Dr. Reimann.

The study of the effect of different environmental conditions on the biological properties of the pneumococcus undertaken last year by Dr. Dahl has been continued. Dr. Dahl had observed that when a virulent pneumococcus was repeatedly grown over a long period of time in homologous serum, or in bile, or even in heterologous serum or in bouillon, that the virulence was markedly diminished and the organisms lost their strict type specificity, and that the antigenic properties were greatly modified. These changes were

particularly marked with the strains treated with homologous serum and bile. The effort has now been made to restore the original specificity and virulence of these modified cultures. It has been possible by repeated passages through mice to cause the strains which were modified by growth in plain bouillon, heterologous serum and homologous serum to regain the specific properties which they originally possessed. The strain treated with bile, on the other hand, has thus far remained refractory. Even after 100 passages through mice this strain is only moderately virulent, and the specific agglutinability is very faint. Moreover, the cocci are still highly resistant to the action of bile.

Several recent investigators have observed that when certain organisms (Shiga bacilli, streptococci and pneumococci) are grown under adverse conditions certain changes are produced in individual organisms and that when these strains are plated, colonies of different types are seen. The differences in the colonies are so great that the different types can be differentiated macroscopically. The organisms isolated from the different types of colonies are also found to vary markedly in virulence, type specificity and antigenic properties.

This phenomenon has been studied in a culture of pneumococcus which had been grown 240 times in plain bouillon. When this culture is grown on agar plates colonies of two kinds have been found. Also the pneumococci isolated from a colony of one type differ markedly in virulence, and specific immune reactions from the pneumococci isolated from a colony of the other type.

The studies so far made indicate that under certain conditions pneumococci may suffer a loss or modification of the function upon which the specificity depends. In no instance so far studied, however, has any organ-

ism been found to acquire any new specific function.

Changes in the Blood in Pneumonia.

Dr. Reimann.

Blood Platelet Count, Corpuscle Volume and Sedimentation Time of Red Corpuscles. An improved technique recently devised for counting blood platelets has been employed in studying the variation in the number of blood platelets during the course of pneumonia. At the same time the sedimentation time of the red blood corpuscles and the relation of corpuscle volume to plasma volume have been studied.

Eight cases have so far been studied during the entire course of the disease and it has been found in each instance that the platelet count is diminished during the acute stages. At the time of crisis or lysis, or shortly after, the number of platelets increases, and in 7 to 9 days the number is double the normal. The count returns to the normal limits in about 2 weeks. If serum disease occurs the number again diminishes.

The volume of red blood corpuscles is diminished during the fever out of proportion to the decrease in the number of red blood corpuscles. The volume returns to normal in about 7 to 14 days after the crisis.

The speed of sedimentation of red blood corpuscles is also markedly increased during the fever, and diminishes very gradually, until it returns to the normal time in about 14 days.

Further studies are in progress to determine the significance of these changes.

The Inhibitory Action of Serum and Leucocyte Mixture on the Growth of Pneumococci.

Dr. Sia.

As is well known, under ordinary conditions pneumococci will grow readily in blood, even in the blood of animals immune to infection. Yet when

inoculated into the blood stream of animals resistant to infection with pneumococci the pneumococci fail to grow. The exact method of this destructive or inhibitory action of the fluids of the body has been much studied, but the technique heretofore employed has not permitted accurate quantitative results to be obtained. It is generally believed that phagocytosis plays an important part in this phenomenon, but other factors may also be concerned.

Dr. Robertson and Dr. Sia, working in Pekin last year, devised a method for studying the destructive action of blood, or more particularly, of serum leucocyte mixtures. The method consists in employing known quantities of serum and washed leucocytes in small glass tubes seeded with varying numbers of pneumococci. The tubes are then sealed with paraffined corks and attached to an agitating apparatus placed in the incubator. A constant and thorough mixing of leucocytes and microorganisms was thereby obtained during incubation.

Employing this method Robertson and Sia have found that when varying numbers of pneumococcus are inoculated into tubes containing constant quantities of a mixture of serum and leucocytes from animals resistant to pneumococcus infection, such as cats, dogs and pigeons, growth fails to occur in the tubes containing considerable numbers of bacteria. When, on the other hand, similar inoculations are made into tubes containing mixtures of serum and leucocytes from susceptible animals, such as rabbits and guinea pigs, growth occurs in all the tubes even in the tubes containing the smallest number of organisms. The results obtained were consistent and definite, and apparently, show that natural immunity, just as artificial immunity, depends on the inhibition or prevention of the growth of pneumococci by the action of body fluids. They have shown by this method that the immune property resides in the serum

Dr. Sia has been working in this hospital during the past three months. He has brought with him the apparatus employed for keeping the mixtures in constant motion and he has undertaken to determine the role which the so-called soluble substance plays in the phenomenon of growth inhibition. He has found that the soluble substance, even in dilutions as high as 1:2,500,000, exerts a definite effect in increasing the inhibitory action of serum leucocyte mixtures. Further studies have shown that this effect is not due entirely to a modification of the bacteria, but is in part the result of an injurious action upon the leucocytes.

Also a study with this method is being made of the appearance of growth inhibiting properties in the blood of patients suffering from pneumonia. It has been found that at the time of crisis and for a short time thereafter the blood of patients suffering from pneumonia acquires very marked growth inhibiting properties. A series of cases is now being studied and curves of growth inhibiting power of the blood are being made for the purpose of determining the time at which this power is at its height and when it disappears.

Oxygen Chamber.

Dr. Binger and Dr. Brow.

The new oxygen chamber has been in use since the first of the year. For simplicity and economy of operation it has proved very satisfactory. Owing to the scarcity of severe cases of pneumonia this year only a small number of cases have been treated in the chamber. The general policy is to use the chamber for cases in which the prognosis is unfavorable and in which there is evidence of oxygen want. Type I cases are ordinarily not treated in the chamber but with serum. The observations made this year confirm those previously obtained, namely, that in patients with cyanosis the breathing of

an atmosphere containing an increased percentage of oxygen affords to the patient a very definite sense of relief and increased comfort, the anoxemia is diminished or relieved, and the cyanosis becomes much less. It is still too soon to make any definite statements in regard to the effect on mortality rate. A description of the chamber is being prepared for publication.

Dr. Binger in conjunction with Dr. Barach of the Presbyterian Hospital has prepared an oxygen bed tent for use in hospitals which cannot afford to construct chanbers and possibly for use in homes. The method of oxygen and CO_2 analyses used in the chamber have been adapted to the tent and a system of air purification and cooling has been constructed which keeps the air in the tent comfortable. This tent has been used satisfactorily by Dr. Barach on several cases. A description of the tent will be published soon by Drs. Binger and Barach.

Animal Experiments.

Dr. Binger and Dr. Brow.

In connection with the work on anoxemia and oxygen therapy in pneumonia, a series of animal experiments are being conducted with the object of inquiring into the mechanism by which rapid breathing and anoxemia are produced.

The teaching of Haldane and his pupils has been that in pneumonia there occurs an unequal distribution of air in the lungs permitting part of the blood to return to the systemic circulation in a state of oxygen unsaturation. This results in oxygen want in the tissues. As a result of the deficient oxygen supply in the central nervous system rapid and shallow breathing is initiated, and this type of breathing is ineffective for the proper ventilation of the lungs and aeration of the blood. A vicious circle is thus established: anoxemia resulting in rapid and shallow breathing; rapid and shallow breathing resulting in anoxemia.

In these experiments this problem is being studied further. By interfering with the pulmonary circulation in dogs severe degrees of anoxemia have been produced and respiratory rates 10 or more times the normal. It is now possible to evaluate the several factors involved in the production of anoxemia and to put to a test the theoretical generalizations laid down by Lundsgaard and Van Slyke in their recent monograph on cyanosis.

The essential findings are as follows. Certain irritant substances (chlorine water, ammonia) injected into the lung by passing a fine catheter down the trachea of dogs produce very rapid respiratory rates. This rapid rate can be immediately stopped by section of the vagus nerves. A method of "physiologically" sectioning the nerves has been found. This consists in passing a silver-plated tube under the nerves and allowing cold brine (-5°C) to flow through the tube. The cold inhibits vagal impulses. After the nerves are allowed to thaw they are found again to transmit impulses.

Certain specific capillary poisons (histamine, tyramine) behave similarly to the irritant substances in producing rapid breathing through vagal paths. It was believed that lesions of the pulmonary capillaries were responsible for this phenomenon. For this reason Dr. Binger and Dr. Brow (following some observations made by Dunn during the war) injected a suspension of potato starch intravenously into dogs and observed the onset of extremely rapid rates of respirations -often as high as 150 per minute or about 10 times the initial rate. This, too, could be reduced to about the normal rate by vagal freezing.

To determine whether the origin of these impulses was actually in the capillaries Drs. Binger and Brow have injected progressively larger particles into the venous system of dogs, beginning with poppy seeds, then rape seeds and, finally, radish seeds. It is of interest that a given number of seeds of any one kind may be injected without apparent effect. As soon as a

larger number of seeds are injected, however, rapid breathing is precipitated. In each one of these experiments careful studies have been made of the oxygen content and capacity of the arterial blood and of its CO_2 content. All of those experiments in which obstruction of the pulmonary circulation has been produced have been accompanied not only by accelerated respiratory rates but by marked and progressive anoxemia of the arterial blood. The anoxemia is apparently independent of the respiratory rate, but it is in some still unexplained manner related to the circulatory obstruction in the lung. This last fact was established by freezing the vagi, maintaining a slow respiratory rate and then injecting the seeds. Such an experiment was accompanied by just as great a reduction in O_2 saturation of arterial blood as an experiment in which the rapid rate was allowed to supervene. It has been found, too, that oxygen administration to these dogs abolishes the anoxemia and restores the respiratory rate to its initial level. So it may be concluded that the accelerated rate results from anoxemia - but the anoxemia is not the result, primarily at least, of rapid breathing. The cause of anoxemia which follows obstruction in the pulmonary circulation is not yet certain.

The striking analogy between dogs whose pulmonary circulation has been obstructed and cases of lobar pneumonia has frequently impressed Drs. Binger and Brow. In both there are rapid respirations, anoxemia, fall of arterial pressure, dilatation of the heart, and in both oxygen inhalations abolish the anoxemia and reduce the accelerated respirations. Recently lung volume changes in those dogs resulting from the obstructed circulation have been found. This is another point of analogy with pneumonia.

The work has been accompanied by very careful pathological and histological studies made by Dr. Branch. He has prepared Barium gelatin injection specimens of the dogs' lungs which when X-rayed or cleared show the distribution of the circulatory obstruction.

Experiments in Breathing.

Dr. Davies.

Experiments are in progress with the Haldane blood gas apparatus in the direction of improving the technique and attempting to reconcile the differences between results obtained by this method and those of the Van Slyke method. It is expected that shortly it will be possible to determine oxygen saturation, total oxygen capacity and carbon dioxide content upon a single sample of 2 cc. of blood. The object of this work is to develop a simple technique together with an inexpensive form of apparatus for routine clinical blood gas analysis.

In conjunction with Drs. Binger and Brow experiments are in progress to determine the respiratory response to varying concentrations of carbon dioxide in the inspired air, and to determine the effects of varying oxygen percentage upon this response. The object of these experiments is to establish normals in order to compare the effects in cases of respiratory disease, especially pulmonary emphysema and lobar pneumonia. It has already been found in the individuals so far investigated that the response varies with different individuals. It also varies in a given individual in different postures and with different oxygen concentrations.

A case of pulmonary emphysema is being investigated as regards lung volume, blood gas and acid-base balance. It is expected that other cases of this disease may shortly be available for similar studies. The object of these investigations is to endeavor to throw some light upon the pathological physiology of this remarkable condition and especially to explain why cases with emphysema can tolerate higher concentration of $C\Theta_2$ in the inspired air than normal individuals.

In conjunction with Drs. Hastings and Murray an experiment was performed to determine the effects of extreme local asphyxia, produced by stasis,

upon the gas CC and acid-base balance of the blood. It is hoped that experiments of this nature may throw some light upon certain of the phenomena resulting from peripheral stasis in cardiac decompensation, and also help to elucidate the means whereby the blood and tissues endeavor to maintain their normal acid-base equilibrium under extreme conditions.

Pathological Laboratory.

Dr. Arnold C. Branch.

During the past six months there have been 11 cases autopsied. 2 of these were from the respiratory service, 1 from the rheumatic, 3 from the cardiac, and 5 from the nephritic. They include:

1 case of lobar pneumonia
1 case of bronchopneumonia (both Pneumococcus group IV).

1 case of rheumatic endocarditis
2 cases of chronic valvular disease
1 case of syphilitic aortitis and aneurysm

3 cases of chronic nephritis,
1 case of chronic nephrosis in a horseshoe kidney
1 case of hemochromatosis.

The last case, in which there was present a carcinoma of the liver with malignant thrombosis of the portal vein, was treated with insulin. This case was of much interest and a full report of the clinical and pathological features is being written by Dr. Branch and Dr. Salvesen.

Dr. Branch has also been engaged in completing the study of the lesions produced in mice by exposing them to atmospheres containing pneumococci in suspension. (See report of Dr. Stillman's work).

In addition, a series of rabbits and rats are being fed on high protein diets with a view to confirming Newburgh's work on the production of kidney lesions by diets high in proteins. Should these experiments prove confirmatory, it is expected to feed animals with tyramine, a product of decomposition of tyrosine in the large gut, as a possible means of producing

chronic nephritis.

The lungs of the series of dogs being experimented on by Drs Binger and Brow have been examined pathologically. Besides histological examination a series of Spalteholz preparations have been made of arterial injections. (See report of Dr. Binger's work).

Report on a Case of Relapsing Trypanosomiasis Treated by Tryparsamide.
Dr. H. J. Morgan.

In connection with the work of Drs. Brown and Pearce concerning the treatment of trypanosomiasis with tryparsamide a brief report of a case of sleeping sickness, or trypanosomiasis, recently treated in the hospital may be of interest, especially since the case offered an opportunity for comparing the therapeutic effect of Bayer 205, a German preparation of unknown composition, with the effect of tryparsamide.

The patient was a woman who contracted the disease in the Belgian Congo in1919. After her return to this country in 1922 trypanosomes were found in the blood and in the cerebrospinal fluid. She went to England where she was given 10 doses of Bayer 205. Definite improvement resulted, and the case was included among the 10 cases reported as "cured" by Manson Barr. On her return to this country in December 1922 she seemed well, but in January, 1923, a relapse of all the old symptoms occurred, although no trypanosomes could be demonstrated in the blood or spinal fluid. She was admitted to this hospital for treatment with tryparsamide and over a period of two months 10 doses of tryparsamide, varying in size from 2 to 3 gms. were administered. Very prompt subjective and objective improvement resulted. She became able to walk, gained 10 kilos. in weight, and, except for a trace of globulin in the cerebrospinal fluid and a very slightly increased cell count, no subjective

or objective manifestations of the disease remained. She has returned to the hospital on several occasions for observation and treatment and has remained very well up to the present. The case is of interest in showing the effect of tryparsamide in a patient in whom a relapse of the symptoms occurred following the administration of Bayer 205.

RUFUS COLE.