Report of Dr. Avery with Drs. Stillman, Goebel, Dubos, Francis, Kelly, Babers, Goodner, and Alloway.

I. The decomposition of the Capsular Polysaccharide of Type III Pneumococcus by a Bacterial Enzyme.
   1. Methods for obtaining potent, non-toxin preparations of the enzyme.
   2. The protective action of the specific enzyme against Type III Pneumococcus infection in mice.

II. Isolation of other microorganisms decomposing the capsular polysaccharides of different types of Pneumococcus.

III. Chemo-immunological studies on Carbohydrates.
   1. The determination of the molecular size of the capsular polysaccharide of Type III Pneumococcus.
   2. The specific carbohydrate of two strains of Pfeiffer's bacillus.
   3. The somatic carbohydrate of Pneumococcus.
   4. Studies on synthetic carbohydrate derivatives.
      a) Synthesis of ~~x~~ para-amino phenol glucoside.
      b) Glucuronic acid.

IV. Chemical Nature of Type Specific, Capsular antigen of Pneumococcus.

V. Significance of the Skin Test as a guide to Serum Therapy in Pneumonia.

VI. Studies on Natural Resistance and the Immunity induced by R pneumococci.

VII. Principles Governing the Precipitin and Agglutinin reactions with Pneumococcus.

VIII. Studies on Epidemiology of Pneumonia.

1. Antibody response to immunization by different routes.

2. Production of experimental pneumonia in animals.

IX. Antipneumococcus Immune Reactions of Normal Hog Serum.

X. Pathogenesis of Experimental Intradermal Pneumococcus Infection.

1. Intradermal infection with a rabbit virulent Type III Pneumococcus.

XI. Studies on the Transformation of the Specific Types of Pneumococcus.

XII. Significance of Oxidation-Reduction Processes in Bacterial Growth.

A. Purification of a brand of commercial peptone.

B. Preparation of peptones from pure proteins.

XIII. Publications.

I. The decomposition of the capsular polysaccharide of Type III Pneumococcus by a bacterial enzyme. (Drs. Avery and Dubos). A microorganism has been isolated, and from it an enzyme extracted, which decomposes the purified capsular polysaccharide obtained from Type III Pneumococcus. This microorganism, a pleomorphic aerobic bacillus, was isolated from a soil rich in hemicelluloses under-going decomposition, by the use of a synthetic mineral medium containing the capsular polysaccharide as sole source of carbon. As already described in a previous report, the endocellular enzyme extracted from this microorganism exhibits a remarkable specificity in its action against the capsular polysaccharide of Type III Pneumococcus.

The work of this year may be conveniently described under two main headings -

1. Attempts at obtaining potent and non-toxic enzyme preparations.

2. Protective action of a specific enzyme against Type III Pneumococcus infection in mice.

Before going on with a description of these studies, it may be worth while to state that the work has been controlled by a rapid and fairly accurate method of titration of the potency of the enzyme preparations in vitro; this method, based on the existence of a quantitative relationship between amount of substrate decomposed and amount of enzyme used, consists in determining the amount of cap-

sular polysaccharide decomposed by a definite amount of enzyme preparation.

1. Attempts at obtaining potent and non-toxic enzyme preparations. The amounts of specific enzyme obtained from a culture of the bacillus decomposing the capsular polysaccharide of Type III Pneumococcus is not a direct function of the number of cells from which the enzyme is extracted. It is conditioned by a series of factors among which the following have been recognized:-

A. Past history of the culture.

B. Composition of the culture medium, with special reference to - a) The presence or absence of the capsular polysaccharide in the medium; b) The presence or absence of substances acting as a more available source of energy for the bacillus: c) The form in which nitrogen is supplied in the medium; d) the presence of catalysts affecting the rate of decomposition of the capsular polysaccharide; and e) The presence of substances retarding autolysis of the cells.

C. Environmental factors, especially - temperature and conditions of aeration.

In brief, the following points must be observed in order to obtain a maximum yield of enzyme. The culture must be maintained at a high degree of specific activity by continued passages in the specific synthetic medium containing the capsular polysaccharide as sole source of energy. The enzyme is usually not formed, or only in small amounts, un-

less the capsular polysaccharide (or the aldobionic acid derived from it), is present in the medium. However, the concentration of capsular polysaccharide must not be too high; it must be such that the decomposition is completed in 2-3 days at the most. This condition may be accounted for as follows:- The enzyme can be demonstrated free in the medium, as soon as the cells begin to age. In case there is still some polysaccharide undecomposed at this time, the free enzyme decomposes the residual polysaccharide and is therefore neutralized without contributing in any way to the anabolic processes of the cell. This results of course in a loss of both enzyme and capsular polysaccharide. This loss may be reduced in two ways: - a) by increasing the rate of bacterial growth (use of a large inoculum, presence of enough nitrogen, proper conditions of aeration, and the addition of growth catalysts, such as soil extract and yeast extract), and by retarding autolysis and thus preventing the liberation of the enzyme (addition of yeast extract to the medium). Under these conditions, the yield of enzyme has been increased almost tenfold over that previously obtained.

Unfortunately some of the new preparations have exhibited a definite primary toxicity. The production of non-toxic preparations is being attempted along three different lines:- a) modification of the medium used for the growth of the bacillus; and b) modification of the process of extraction of the enzyme; and c) by purification of the

enzyme preparations.

2. Protective Action of the Specific Enzyme on Type III Pneumococcus Infection in Mice. The earlier studies comprised observations on the action of the enzyme on the capsular sugar of Pneumococcus removed and separated from the bacterial cells. In the natural state in which this specific polysaccharide exists, it forms the capsular structure which determines the antigenic and serological specificity of the cell as a whole, and conditions its power to invade and multiply in animal body. It was of special interest, therefore, to determine what effect the specific enzyme would have upon the encapsulated forms of Type III pneumococcus growing *in vitro* and *in vivo*:- whether in a medium containing the active enzyme, the pneumococci would fail to grow at all or would grow merely deprived of their capsules; whether in the body of a susceptible animal, the administration of the enzyme would in any way modify the course of experimental infection with virulent Type III pneumococci. The results of these studies form the subject matter of a paper which is now in manuscript form.

Briefly, the work shows that, when a sterile extract of the enzyme is added to a culture medium seeded with Type III pneumococci, growth occurs but the bacteria are deprived of their capsules; they are no longer specifically agglutinable, and the soluble specific substance is no longer demonstrable in the culture fluid. Experiments of this

kind have shown quite clearly that the enzyme by itself is neither bactericidal nor bacteriolytic; that by decomposing the specific carbohydrate, it merely strips the pneumococci of their capsules without impairing the viability of the bacteria. Moreover, the action of the enzyme on the living cell does not result in a loss of the function of elaborating the capsular substance, since pneumococci so treated, regain their capsules and continue to elaborate the soluble specific substance when transferred to fresh medium free of the enzyme.

In more recent studies it has been found that the enzyme has a distinct protective action in the animal body against infection with virulent Type III pneumococci. Mice receiving a single intraperitoneal injection of an active preparation of the enzyme may survive infection with a million times the fatal number of virulent organisms. The protection afforded by the enzyme is type-specific; just as in the test tubes the enzyme acts only on the Type III polysaccharide, so in the animal body it is effective only against infection with Type III pneumococcus. A protocol of an experiment illustrating the degree and the specificity of the protective action of the enzyme in mice is given in Table I.

T a b l e I.

Specificity of the Protective Action of Enzyme.

Infecting dose of Pneumococcus	Enzyme (lot 4a) 0.5cc.			No Enzyme		
	Type I	Type II	Type III	Virulence controls		
				Type I	II	III
cc.						
.1	-	-	S	-	-	-
.01	-	-	S	-	-	-
.001	-	-	S	-	-	-
.0001	D20	D34	S	-	-	-
.00001	D24	D34	S	D22	D36	D34
.000001	D34	D34	S	D34	D36	D34
.0000001	-	-	-	D34	S20	D72

S = Survived. D = Death of animal; the numeral indicates the number of hours before death, or the time at which the animals were found dead. - = Not done.

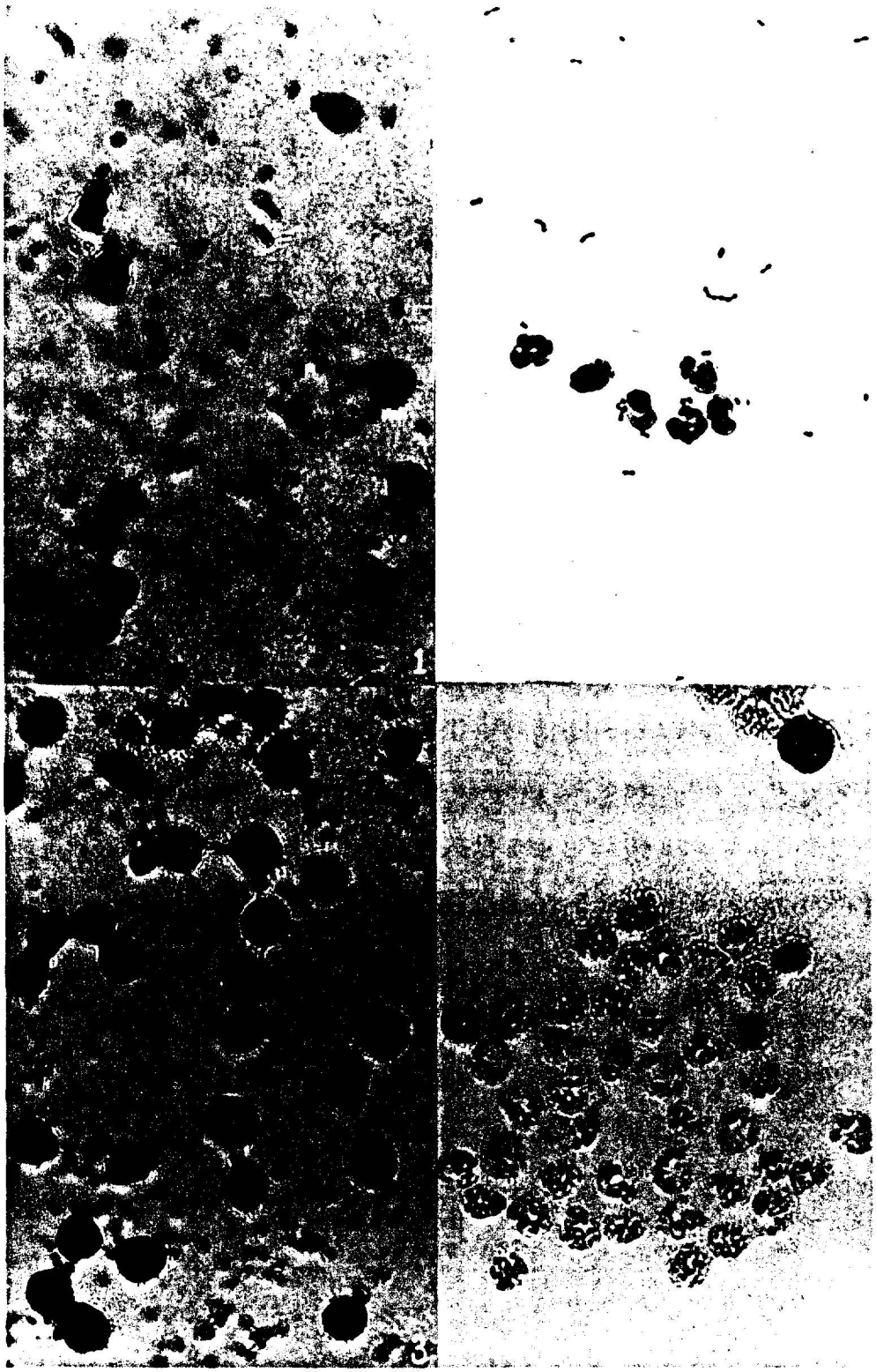
The active principle responsible for the protection of mice is destroyed by exposure to 70° C. for ten minutes. The inactivation by heat of the protective power for mice of this enzyme, parallels the loss of its activity when tested *in vitro* after exposure to temperatures of 60° C. or higher. The fact that heat destroys the power of the enzyme to act either *in vitro* or *in vivo*, justifies the assumption that the same principle is involved in the mechanism of both reactions.

Repeated tests have shown that within the limits of the reaction-capacity of the mouse, the protective action of the enzyme is a function of the concentration of the active principle in any given preparation; and further, that the protection afforded by different preparations bears a

definite relation to their activity in decomposing a known quantity of the capsular polysaccharide in vitro. The evidence is that, to be effective in animal protection, the concentration of enzyme in the body must be maintained in excess so that the rate of decomposition of the capsular substance is always greater than the rate of its production by the living bacteria.

Experimental evidence indicates that in mice the enzyme has a curative action when administered in the course of an infection already established at the time of treatment. A single injection of enzyme as late as 18 hours after the onset of infection has brought about the recovery of mice infected with a dose of virulent pneumococci 100 times greater than that fatal for the untreated controls. It must be borne in mind, however, that the curative action of the enzyme suffers the limitations imposed by the variations which occur in the cellular response of the host, since phagocytosis is apparently the important process involved in the protection afforded by the enzyme.

A better understanding of the mechanism under-lying the protective action of the enzyme in infected mice has been gained by following the course of the bacteremia by means of blood cultures and by a study of the cellular reactions in the peritoneal exudates of treated and untreated animals. The results of a typical experiment are graphically illustrated in the accompanying photomicrograph (fig.1).



In this experimenta, 12 mice were infected with one million fatal doses of Type III pneumococci. Six of these mice received at the time of infection 0.5 cc. of an active preparation of the enzyme. The other six mice served as untreated controls. At intervals following infection, one mouse of each group was sacrificed and autopsied. Cultures of the heart's blood were made, and stained films of the peritoneal exudate were examined to compare differences in the morphology of the organisms and in the occurrence of phagocytosis in the treated and untreated animals.

Two hours after infection the blood cultures of both the treated and untreated mice were positive. Microscopic examination of the exudate of the control animal at the end of 2 hours shows the presence of many well encapsulated cocci in every field without any evidence of phagocytosis. (fig. 1a). On the other hand, the pneumococci present at this same period in the exudate of the enzyme-treated mouse, (fig. 1b), are smaller in size and devoid of capsules; only the naked cells are visible and many of these have already been taken up by the leucocytes. At the end of four hours, the bacteria are more numerous in the peritoneal exudate of the infected control (fig. 1c); they are surrounded by well defined capsules and none are found within the leucocytes; cultures of heart's blood of the untreated animals indicate a progressively increasing bacteremia. In the treated animals at this period, however, (fig. 1d)

only an occasional naked cell is seen outside the leucocytes and frequently at this time and invariably by the fifth hour the organisms are no longer demonstrable in the blood.

The evidence obtained from repeated experiments of this nature supports the view that the protective action of the enzyme lies in its capacity to decompose the capsular polysaccharide of Pneumococcus Type III. The process of decapsulation, brought about by the direct action of the enzyme, strips the bacteria of an important defense mechanism and thereby exposes their naked, unprotected bodies to direct attack by the phagocytes of the host. In this sense the action of the enzymes may be said to initiate phagocytosis; not, as in the case of antibodies, by specifically sensitizing the bacteria, but by the process of decapsulation. In the former instance, the reaction is an immunological one, whereby the specific substance of the capsule is altered by union with the type-specific antibodies; in the latter case, the reaction is a chemical one in which the capsular polysaccharide itself is decomposed by the enzyme. Although the mode of action is different in each instance, the end result, so far as the fate of the pneumococcus is concerned, is the same in both instances.

The enzyme, like the type-specific antibody, serves to initiate a protective reaction, the completion of which, however, is ultimately dependent for its successful issue upon the effective cellular response of the host. This study

suggests that the capsule - long recognized as a defense mechanism of virulent bacteria - is a decisive factor in determining the fate of pneumococci in the animal body, and that this structure is vulnerable to attack by specific agents other than antibodies.

II. Isolation of other microorganisms decomposing the capsular polysaccharides of different types of Pneumococcus. (Dr. Dubos). The search for other microorganisms decomposing the specific polysaccharides of Pneumococcus types is being continued.

A new spore forming, Gram positive, thermophilic bacillus, decomposing the capsular polysaccharide of Type III Pneumococcus, has just been obtained from horse manure. Nothing is known as yet of the biochemical properties of this organism except that it decomposes the Type III polysaccharide when incubated at 50° C.

From a corn cob compost, there has been isolated an unidentified microorganism, probably belonging to the Mucorales, which decomposes the Type II capsular polysaccharide. This organism does not attack the capsular polysaccharides of Type I and Type III Pneumococcus.

It may be recalled that the capsular polysaccharide of Type II Pneumococcus exhibits the same serological reactions as that of Type B Friedländer bacillus. It is a remarkable fact that the same microorganism which specifically decomposes the Type II polysaccharide of Pneumococcus,

also decomposes the immunologically related capsular substance of Type B Friedländer bacillus. These observations again confirm the fact that the capsular polysaccharides determine the specificity of these serological types.

III. Chemo-immunological Studies on Carbohydrates. (Dr. Goebel and Mr. Babers). 1. Determination of the molecular size of the capsular polysaccharide of Type III Pneumococcus. A preliminary account of the determination of the molecular size of the specific polysaccharide of Type III Pneumococcus was given in the last report. This work has since been completed. The diffusion coefficient of the sodium salt of the specific carbohydrate has been found to be 0.0415 Cm.^{2} per day. From this value the radius of the molecule has been calculated as 2.975×10^{-7} cm. and subsequently a molecular weight of 118,000 has been ascertained. The molecular weight of hemoglobin and of the carbohydrate from ovomcoid were determined in control experiments. Values of 67,200 and of 2,200 respectively, were found. These numerical values are in agreement with the accepted values of other investigators, reported in the literature.

2. The specific carbohydrate of two strains of Pfeiffer's bacillus. The specific carbohydrate from a strain of Type A Pfeiffer's bacillus has been isolated from cultures, grown on dextrose-agar. The medthod of isolation was similar to that which has been used in the preparation of other bacterial polysaccharides. Three preparations have been secur-

ed which react with homologous immune rabbit serum in dilutions of 1:2,000,000. The carbohydrate has a levo-rotation of -32°, it is acidic, and in the present state of purification contains only slight traces of nitrogenous constituents.

The carbohydrate from a strain of Type B Pfeiffer's bacillus is also being studied. Thus far a material has been secured which possesses only a fourth of the serological activity of that of the Type A strain. This carbohydrate appears to be much less stable than that of Type A. Both the A and B types of Pfeiffer's bacillus elaborate only small quantities of specific polysaccharides, about one-tenth the quantities obtained from Pneumococcus. A more detailed account awaits the preparation of sufficient quantities of the materials for chemical investigation.

3. The somatic carbohydrate of Pneumococcus. The "C" substance, or species-specific somatic carbohydrate of Pneumococcus has been identified as a nitrogenous polysaccharide which yields 30 per cent of reducing sugars, calculated as glucose, on hydrolysis. At the suggestion of Dr. Francis, a phosphorous analysis was made on this substance by Dr. Heidelberger at the Presbyterian Hospital. The carbohydrate contains organically bound phosphorous to the extent of approximately 3.3 per cent. We have also found this material to contain about 5 per cent of nitrogen. The carbohydrate is hydrolyzed by nitrous acid in the cold, with an accompanying loss in specificity. We have attempted to split

off the organic phosphorous of this carbohydrate by means of a powerful phosphatase, but the enzyme causes no liberation of inorganic phosphorous. At present sufficient quantities of this material are being prepared for Dr. Francis in order that he may carry out certain clinical studies.

4. Chemo-immunological studies on synthetic carbohydrate derivatives. In previous reports we have described the method for the preparation of synthetic carbohydrate (hexose)-protein antigens, as well as the preparation of a polysaccharide-protein antigen which elicit specific immune responses when injected into animals. From these studies we have learned first, that carbohydrates, both simple and complex, can be rendered antigenic when they are coupled to a protein, and second, that the specific immune response (in the case of hexoses) is determined by the configuration of the hexose molecule. In the case of large molecules, such as the specifically reacting bacterial polysaccharides, the ultimate immune response is such that the induced antibody will not only agglutinate microorganisms from which the carbohydrate was derived, but will actually confer passive protection on mice infected with an homologous type of organism. We are inclined to believe that in order to secure immunity against a disease in which the chief immune response is dependent upon the elaboration of anticarbohydrate antibodies, it is necessary only to have as the antigenic agent, a combined carbohydrate, of an exact and correct chemical confi-

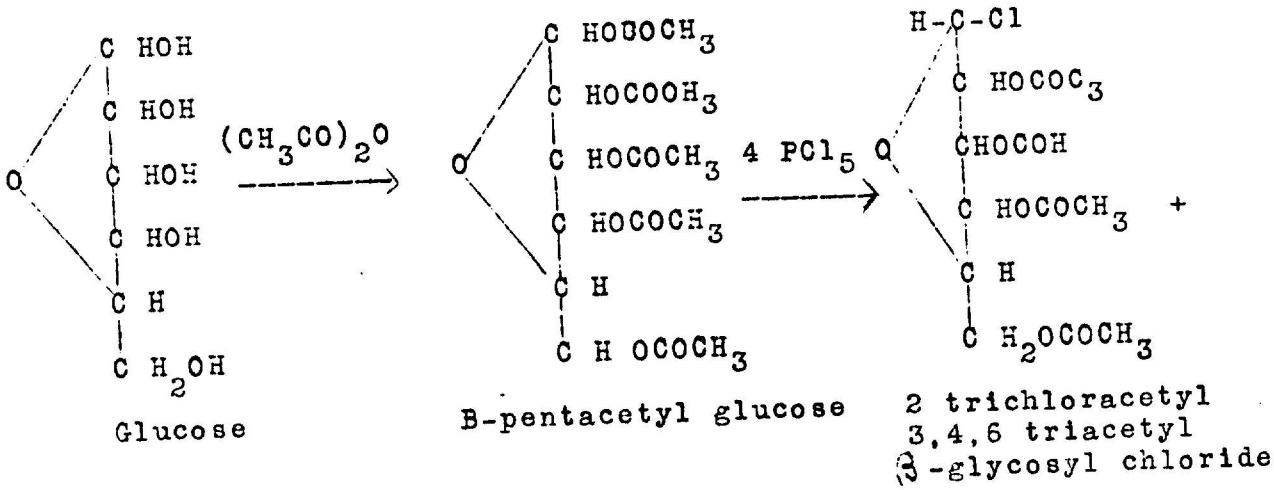
guration, capable of stimulating the formation of these antibodies.

In a molecule as complex as that of Type III Pneumococcus polysaccharide there are naturally many factors which enter into the orientation of its ultimate specificity. We know, for example, that the molecule is studded with highly polar carboxyl groups. We know that there are three free hydroxyl groups per unit of hexose, and we know that the molecule is a polymer of an aldobionic acid (glucurono-glucose). We do not know, however, the mode of linkage of one aldobionic acid unit to the next; we do not know whether the glucurono-glucose is an α or β glucuronoside, nor do we even know if an α sugar can give rise to a different antibody than its β homologoue.

However, these fundamental problems in the understanding of the specificity of immunological reactions in the case of carbohydrates are not entirely unapproachable, for we have at our disposal methods based on synthetic chemistry, whereby we can analyze the influence, not only of intermolecular stereochemical relationships, but the influence of polar groups in the carbohydrate molecule as well.

a) The synthesis of α para-amino phenol glucoside. We have demonstrated in previous work that the alteration of the stereo-chemical configuration of one carbon atom in different hexoses, suffices to elicit totally different and specific antibody response. Our next problem is to

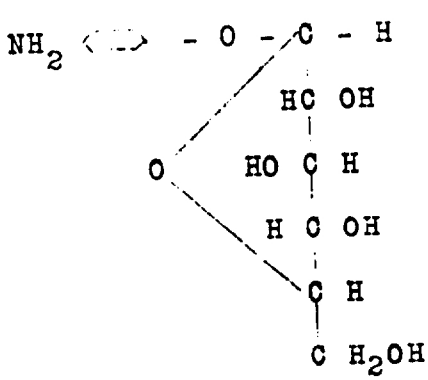
ascertain whether the α and β glucosides of the same hexose would give rise to separate and distinct antibodies, or merely to antibodies showing but slight differences. We have already synthesized the β-p-nitro phenol glucoside of glucose. The homologous α glucoside has been synthesized in the following manner.



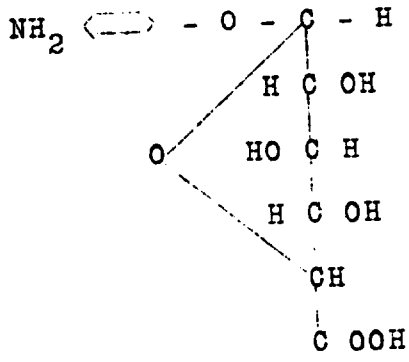
The nitro phenol glucoside has not as yet been reduced to the amino homologue. The α nitro phenol glucoside analyses correctly for carbon and hydrogen. It melts at 220° and has an optical rotation of +233.9° whereas its isomer, the β-glucoside, melts at 164° and rotates the plane of polarized light - 79.6°. We intend to reduce the α compound catlytically, to couple it to protein and to immunize animals and finally to compare the antibody thus produced with those obtained by immunization with the β-glucoside-protein complex.

b) Studies on glucuronic acid. We have spoken above of the occurrence of glucuronic acid in the Type III Pneumococcus specific polysaccharide, and we have indicated that the carboxyl groups remain free in the intact molecule. Not only have we found glucuronic acid in this specific carbohydrate, but we have found it in every bacterial polysaccharide investigated in our laboratory. Furthermore, we have found it in gum acacia, and we have shown that gum acacia will react with antipneumococcus Types II and III sera in dilutions as high as 1:6,000,000 of the carbohydrate. It seems, therefore, that this important constituent of so many specific polysaccharides must play an important immunological role. It is now our belief that the highly polar carboxyl group of glucuronic acid, and its relationship to other polar groups in the carbohydrate molecule, exert a strong influence in the immune response. In order to test this conception, we have decided to determine whether there are

differences in the antibodies produced by immunization with protein complexes of β-p-amino phenol glucoside of glucose and those of β-p-amino phenol glucoside of glucuronic acid.



β-para amino phenol glucoside



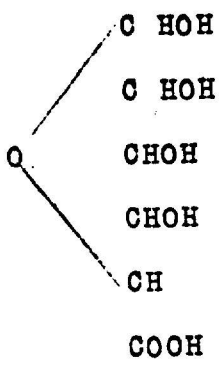
β-para amino phenol glucoside of glucuronic acid

From these two formulae one may see that the stereochemical relationship of these two molecules are exactly alike. They differ only in that the symmetrical sixth carbon atom of the glucose glucoside, a primary alcohol group, is in the form of a polar carboxyl group in the glucuronoside. Since the other atomic groupings of these two compounds are exactly alike, save in this one respect, it is possible to determine directly the influence on the immune response of the substitution of an acid grouping in a hexose molecule.

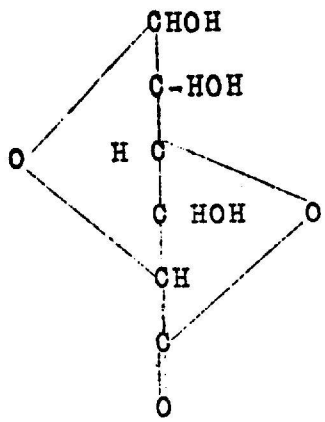
The problem of synthesizing the para-amino phenol glucoside of glucuronic acid is by no means simple. It is difficult to secure large quantities of the sugar acid, and the chemistry of the acetyl and halogen acetyl derivatives from which these glucosides can be prepared, is unknown.

The method of procedure is as follows:- Glucuronic acid was prepared by feeding 5 grams of borneol daily to each of 6 dogs. The animal organism conjugates the borneol and excretes it in the form of a glucuronic acid glucoside which can be quantitatively recovered from the urine as a crystalline zinc salt. Between 20 and 30 grams of zinc borneol glucuronic acid can be recovered daily from 6 dogs. The zinc borneol glucoside is treated with sulfuric acid to split off the zinc, yielding the borneol glucoside of glucuronic acid. The latter is then hydrolyzed with 0.2 normal sulfuric acid, and the insoluble borneol is separated from the solution of glucuronic acid. After removal of the sulfuric acid by $BaCO_3$ the glucuronic acid can be crystallized as a snow white product. The chemical manipulation can be easily carried out, and the ultimate yield of this rare sugar represents nearly 90 per cent of the source material.

The sugar can be converted to its lactone by crystallization from acetic acid.

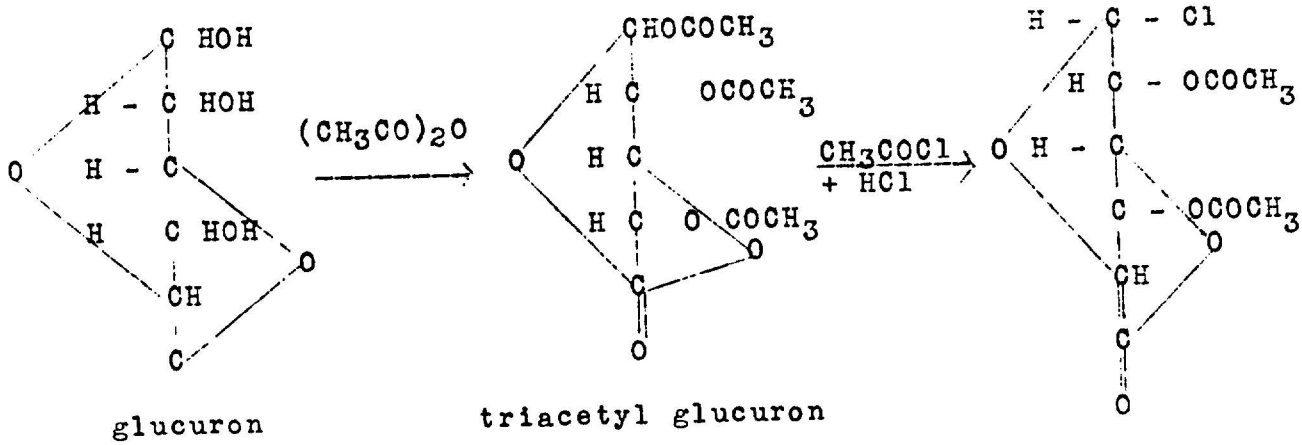


glucuronic acid



glucuron

This derivative has been acetylated and α and β-triacetyl glucuron have been separated by fractional crystallization from alcohol. Neither the α nor the β-triacetyl glucuron will yield a stable bromo derivative, but we have secured from both derivatives a stable diacetyl chloroglucuron.



glucuron — triacetyl glucuron — 1-chlor diacetyl glucuron

Though yet obtained in a crystalline state, these halogen acetyl derivatives give correct analyses. The synthesis has not as yet progressed beyond this point; but the preparation of these two chloro diacetyl glucuron derivatives now make possible the completion of the synthesis of the para amino phenol glucoside of glucuronic acid, which we hope to accomplish in the near future.

IV. The Chemical Nature of the Type-specific Capsular Antigen of Pneumococcus. (Drs. Avery and Goebel).

The importance of determining the chemical nature of the type-specific antigen of Pneumococcus becomes evident when one considers that the protective substances in anti-pneumococcus serum are precisely those antibodies which are produced in response to this particular antigen. By speci-

fic union with these antibodies, the capsular substance of the virulent Pneumococcus bocomes so altered that the bacterial cell as a whole is rendered susceptible to phagocytosis. And, so far as our knowledge at present goes, the therapeutic effect of antipneumococcus serum is largely, if not exclusively, dependent upon the presence of these type-specific antibodies. Indeed, the application of serum therapy in Pneumococcus today rests upon our knowledge of these type-specific relationships. It would appear, therefore, that an understanding of the chemical nature of this important antigenic constituent of the Pneumococcus might contribute much to the improvement and perfecting of methods useful in the production of potent antisera.

Interestingly enough, although the exact nature of this antigen itself eludes us, we have considerable knowledge of one of its component parts, namely, the type-specific capsular polysaccharide. For instance, we know that this specific carbohydrate is given off from the bacteria growing in the focus of disease and circulates in soluble form in the body fluids; that in this form it still retains its capacity to unite with and thus to neutralize the immune substances of the serum; that even in minute amounts it inhibits phagocytosis, and that because of these reactions its presence tends to interfere with the normal processes of recovery from the disease. We know also something of the chemistry and immunological specificity of this capsular substance.

We have succeeded in chemically identifying a polysaccharide in the capsular substance of each of the different types of Pneumococcus and have found in each instance that the carbohydrate is as chemically distinct as it is serologically specific for each type. We have demonstrated the remarkable specificity of these carbohydrates in the phenomenon of anaphylaxis and in the reactions they incite in the skin of convalescents from pneumonia. We have evidence that these specific carbohydrates, when separated from the bacteria, still retain unimpaired the property of combining with antibodies, but lose more or less completely their power to incite the formation of these same antibodies when injected alone into the animal body. This very fact indicates that in their native state, these specific polysaccharides exist in the cell not as haptens but as part of a more complex antigen from which they may be more or less readily dissociated either before or after the injection of the whole antigen into the animal body. The rate and extent to which this dissociation occurs apparently varies with the different types. The theory of antigenic dissociation and its relation to the production of specific sera have been discussed in a preceding report. It is referred to here merely to emphasize the importance of knowing the chemical nature of the type-specific antigen, and to point out again the practical bearing which such knowledge might have upon the problems of active and passive immunity to Pneumococcus infection. Progress in the solution of this problem has been

impeded by two major technical difficulties:- one, the difficulty of extracting the intact antigen in active form because of the ease with which dissociation of the complex occurs; and the other, the difficulty of identifying in such extracts the particular substance or chemo-specific groups which confer antigenicity upon the capsular polysaccharide.

The first of these difficulties has been largely overcome, and the second has been attacked through an indirect but promising approach. Following the leads suggested by the work of Perlzweig and of Day, filtered solutions containing the type-specific antigen in active form have been obtained by extracting the encapsulated cells at an acid reaction with heat. The antigenic activity of the extracts has been tested by injecting them into mice and determining whether the treated animals have acquired specific immunity to infection with pneumococci of the homologous type. In an acid buffer solution (pH 6), the antigen resists heating in a boiling water bath for ten minutes; two injections totaling 0.4 cc. of a solution containing as little as 0.003 mgs. N per cc. have proved effective in immunizing mice against an homologous strain of Type I pneumococcus. The extraction of the antigen, however, is only the first step in the analysis of its chemical nature. Is the type-specific polysaccharide bound to a protein, or to some other substance? Is it even necessary to assume that the effective complex consists of a protein coupled to the carbohydrate? This seems

the most reasonable assumption, especially since we have found that an artificial antigen prepared by the coupling of a diazonium derivative of the Type III polysaccharide with animal protein stimulates in rabbits the formation of type-specific antibodies which agglutinate living pneumococci of the homologous type and protect mice against infection with virulent Type III pneumococci. Despite this analogy, may it not be that the polysaccharide carrying the specific binding groups is but part of a still larger and more complex carbohydrate molecule from which the antigenic groups may be easily split off, leaving the antigenically inert but specifically reactive polysaccharide intact. In the case of the pectins for example, partial hydrolysis suffices to split off pectinic acid from the complex and a polysaccharide then remains as a product of hydrolysis.

As Wells points out, there is theoretically no reason why a carbohydrate should not function as an antigen provided it is colloidal in nature and of sufficient molecular size. However, to assume that the lack of antigenicity of the capsular polysaccharides is attributable to denaturation in the process of isolation is to overlook the fact that the more these substances are chemically purified the more specifically reactive they become and the more closely they conform on chemical analysis to the theoretical values for pure carbohydrates.

The mere prediction of theoretical possibilities

however, is not paramount to the actual identification of the true antigen. The amount of substance required for chemical analysis is large and this is difficult to obtain. Determining whether a given extract contains the effective antigen involves the process of immunization and this requires time. It seemed possible, however, that an indirect approach and one which might furnish a clue as to the nature of the substance to be looked for, might be made by determining if possible, what enzyme or class of enzymes would destroy the antigenicity of a bacterial extract known to contain the whole antigen. Whether for example, the antigenicity of an extract is destroyed by trypsin, or pepsin, etc., or by lipases or nucleases, etc., or whether enzymes, such as pectinase, emulsion, and others which are known to hydrolize partially the more complex carbohydrates would destroy the effective antigen without impairing the specific binding properties of the polysaccharide.

In collaboration with Dr. Goebel this work is now in progress, and the results thus far obtained are promising, but not as yet sufficiently established to warrant presentation at this time.

V. The Significance of the Skin Test with Specific Polysaccharide as a Guide to Serum Therapy in Pneumonia. (Dr. Francis). During the study of the cutaneous reaction of pneumonia patients to the type specific polysaccharides, it was soon observed that all of the cases of Type I pneumonia which

recovered gave a positive reaction to the Type I polysaccharide. The majority of Type I patients received specific serum therapy, while those suffering from infections due to Type II and III Pneumococcus do not. Approximately only 50 per cent of the latter group reacts to the specific soluble substance of the respective types. In none of the patients was this reaction elicited before the onset of recovery, even when type specific therapeutic serum had been administered. Furthermore, in certain instances, when the patient still appeared sick and the problem of additional serum treatment was immediate, a positive skin test was found to indicate recovery and that treatment could be safely discontinued. In the case of individuals, in whom no positive reaction was obtained at any time during the course of the illness, a fatal termination occurred, even though a subsidence of fever, sterilization of the blood of the invading organisms and a comparatively high concentration of serum antibodies were observed. Consequently a negative cutaneous reaction to the polysaccharide was thought to be an indication for continuation of serum therapy.

The study has been extended with the idea of establishing the true value of this reaction as a guide to proper serum therapy. At present, data have been obtained in 35 cases of Type I pneumonia. Four of these were not treated with serum but recovered and gave positive skin reactions; they serve as controls for the cases which received

specific treatment. The results are tabulated below.

	Recovered			Died	
	Total Number	Positive Skin Reaction	Negative Reaction	Positive Skin Reaction	Negative Reaction
Serum Treated	31	25	1*	0	5
Untreated Controls	4	4	0	0	0

*Empyema

From the table it can be seen that of 26 recovered cases which received serum, only one failed to give a positive response to an injection of the specific polysaccharide and in the exception a purulent complication had developed. In the remaining 25, all of whom gave a positive skin test, recovery took place despite such complications as sterile pleural effusions, delayed resolution, furunculosis, and recurrent acute nephritis. In 5 fatal cases no skin reaction was obtained at any time.

The extended observations have, therefore, confirmed the impression that the skin test with type specific polysaccharide is a valuable guide in the serum treatment of pneumonia. Its value lies in the fact that, when positive, it indicates that recovery has begun and serum therapy may be safely discontinued, but a negative respomse is an indication that the administration of serum should be continued. If, in the face of continued serum therapy with definite clinical improvement a negative reaction persists, a suppurative complication should be suspected. If none is found serum therapy should be maintained, although the outlook is unfavorable.

VI. Studies on Natural Resistance and on the Immunity induced in Rabbits by Injections of R Pneumococci. (Dr. Francis). A study is being made of the natural resistances of the rabbit to Type III Pneumococcus and of the form of immunity produced in rabbits by injections of R pneumococci. The method of Robertson and Sia, using serum-leucocyte mixtures, has been shown to be quite sensitive in detecting slight degrees of immunity. For this reason the same method, as well as a modification employing whole heparinized blood, was adopted in the present study.

Although the work is in its early stages, several interesting facts have been revealed. While the normal rabbit is resistant to the rabbit-avirulent strain of Type III Pneumococcus, no pneumococcidal powers against this organism is demonstrable in the animal's blood. Against the R form of Pneumococcus, however, the normal rabbit exhibits the power to inhibit the growth of a large number of organisms. The blood of a rabbit containing anti-R antibodies, produced by immunization with R pneumococci, has an even greater bactericidal power for the R organisms than has the normal animal. In addition, it possesses a moderate but definite capacity to prevent the growth of the avirulent encapsulated Type III Pneumococcus. So far no experiments have been done with the blood of immune rabbits containing type specific antibodies. The state of immunity has been simulated, however, by the addition of type specific horse anti-serum to the blood of the normal rabbit. Under these conditions a

marked pneumococcidal effect on the rabbit avirulent Type III pneumococci is also observed.

Certain conclusions appear justifiable. The normal rabbit, resistant to the avirulent encapsulated Type III Pneumococcus, shows no evidence in the blood of a phagocytic mechanism for its disposal. In the R immune rabbit this capacity has been developed to a moderate degree, while the blood of the animal containing type specific immune bodies has a still greater pneumococcidal power.

VII. The Principles Governing the Agglutinin and Precipitin Reactions. (Dr. Francis). It was observed in the course of precipitin reactions that when a prozone was produced with an excess of soluble specific substance of the pneumococcus, the serum had also lost its capacity to agglutinate the homologous type-specific organism. This observation brought further evidence to indicate the unity of agglutinogen and precipitinogen. Consequently the following experiments were devised in an attempt to correlate the mechanisms of the reactions of agglutination and precipitation.

To an immune serum a suspension of the homologous type specific pneumococcus was added. After agglutination had occurred the compact mass was placed in a dilute concentration of the specific polysaccharide of the same type. Under these circumstances precipitation occurred, with removal of the polysaccharide from solution, indicating that an excess of antibody was attached to the bacterial cells

and was able to unite with additional amounts of antigen, in this case the specific soluble substance. Now, if still more of the free soluble substance was added, the agglutinated organisms became dispersed and remained diffusely suspended in solution. When the bacteria so dispersed were removed from the mixture by centrifugation they were found to be in a normal serological state as shown by the fact that they could be agglutinated again, but only by immune serum for the homologous type of Pneumococcus. Had antibody remained bound to them, the bacteria might conceivably agglutinate spontaneously in heterologous serum, or in salt solution. This, however, was not the case. Similarly, organisms which were added to a serum in which a prozone had been created with specific soluble substance were serologically unaltered.

The results show that the type-specific precipitin and agglutinin reactions are dependent upon the reaction of antibodies with the same substance; in one case, present in the bacterial cell; in the other, chemically purified and separate from the bacteria themselves. The two forms of the type specific substance may be substituted for one another without altering the phase of reaction.

Heidelberger and Kendall recently studied the precipitin reaction quantitatively and concluded that the various phases of reaction could be expressed in terms of the laws of mass action. That is, antibody and specific substance combine in multiple proportions, depending upon the

concentration of the two reagents. The present studies conform qualitatively with theirs, and it seems that the same laws govern both the agglutinin and precipitin reactions.

VIII. Studies on Epidemiology. (Dr. Stillman).

1. Antibody response to immunization by different routes. The work on the epidemiology of pneumonia is being pursued along two lines. Data is being accumulated as to the development of immunity following immunization of rabbits with different types of pneumococci by various routes. The length of time that agglutinins and protective antibodies persist in the serum of rabbits immunized with heat-killed Type I pneumococci has been determined. Agglutinins soon disappeared from the sera of these rabbits. Protective antibodies, on the other hand, could be demonstrated in high concentrations in their sera for prolonged periods. It appears that there is a definite relationship between the total amount of antigen administered and the length of time antibodies may be demonstrated in the blood or rabbits. Furthermore, there is a close relationship between the route of administration of antigen and the character and persistence of the antibody response in rabbits.

2. Production of experimental pneumonia in animals. Work is also in progress on the production of experimental lobar pneumonia in laboratory animals. This has a two-fold object. Once the complicated mechanism of the factors necessary to cause an animal to localize pneumococcus

infection is understood, we will be able to not only treat the disease of lobar pneumonia in man more efficiently but also know better what precautions to observe in order to escape the disease. Although experimental lobar pneumonia has been successfully produced in mice, the various factors entering into the production of pneumonia could not be studied in such a small animal. Ever since Wadsworth claimed to have produced pneumonia in partially immunized rabbits in 1904, it has been assumed that pneumonia could be induced in these animals. It has already been shown that pneumonic consolidation may be produced in partially immunized alcoholized mice by the inhalation method. But we have been unable to cause partially immunized rabbits to localize the infection which develops following inhalation of virulent pneumococci. The inability of rabbits to localize the infection may possibly be due to the anatomy of the rabbits' lung. Pneumococci in rabbits rapidly pass through the pleura and cause empyema and pericarditis. There seems to be no attempt to confine the infection within the lung itself.

IX. The Antipneumococcus Immune Reactions of Normal Hog Serum. (Dr. Kelly). It was reported by Bull and McKee in 1921 that the blood serum of normal chickens when injected into susceptible animals confers a notable degree of passive antipneumococcus immunity. They also showed that the factor responsible for this immunity is associated with serum globulin, and that it is type specific in action.

In 1924 Robertson and Sia by means of serum-leucocyte mixtures were able to demonstrate the presence of naturally occurring antipneumococcus opsonins in the blood of resistant animals. By this technic the serum of normal pigs showed a high content of these opsonins.

Sia in 1929 found that pig serum injected intraperitoneally into mice affords these animals a marked degree of protection against pneumococcus infection. This antipneumococcus protective action was shown by absorption experiments to be type specific in nature.

At variance with the widely accepted theory that natural immunity is dependent solely on the cellular defense of the host, are the two examples, here cited, of a natural immunity to pneumococcus infection that is passively transferable through the blood serum, and that appears to be type-specific in nature.

The present study is an attempt to repeat the experiments of Sia, and to further analyze the antipneumococcus immune reactions of the serum of normal hogs and of other resistant animals. In confirmation of Sia's results it has been found that the serum of normal hogs when injected intraperitoneally confers upon white mice a marked degree of immunity to Pneumococcus, Types I and II. 1 cc., the optimal protective dose of the serum, usually affords protection against 10,000 to 100,000 lethal doses of pneumococcus culture. The serum yields its maximal protective

action when injected four to eight hours before inoculation of the mice with culture. The degree of protection against Type II is consistently higher than that against Type I. The serum gives no protection against pneumococcus infection when injected subcutaneously. Under the same conditions the serum from other animals - rabbit, guinea pig, ox, and sheep, - when injected intraperitoneally, have conferred no protection on mice.

The protective principle of hog serum is associated with the serum globulin obtained by precipitation with ammonium sulphate. Hog serum gradually loses its antipneumococcus protective action on standing, so that after two to four months in the ice-box the protective action is entirely, or almost entirely, lost. At room temperature this loss occurs more rapidly. In a paraffin-lined tube, under a vaseline seal the serum loses its protective power in about the same time as it does when unsealed. The protective action of the serum against Type I is lost usually in shorter time than that against Type II. The protective power is considerably reduced by heating the serum to 56° C. for 30 minutes, and is entirely destroyed by exposure to a temperature of 64° C. for the same time. The protective action when lost, either through heat, or standing, is not restored to the serum by the addition of a small amount of fresh normal serum.

The antipneumococcus protective principle for either Type I or Type II can be specifically absorbed. The

quantity of pneumococci per volume of serum necessary for specific absorption, is small. The absorption requires only a short time for completion. Absorption of the serum with avirulent R pneumococci, and with bacteria other than pneumococci, does not consistently alter the protective power of the serum. Absorption with charcoal and Kieselgur does not appreciably lower the protective power.

Sia in 1922 noted that S pneumococci, when sensitized by large amounts of hog serum, show agglutination after separation from the sensitizing serum by centrifugation. It has been found in the present study that hog serum agglutinates S pneumococci. The agglutinated pneumococci from a firm, thin disc similar to that produced by type specific antipneumococcus serum. In comparison with specific antipneumococcus serum large amounts of hog serum are necessary for demonstration of the agglutination. It is necessary also, except occasionally in case of Type II, to use Gate's technic for this demonstration. The agglutinins for Type II are present in hog serum in greater concentration, than are those for Type I. Similar to the antipneumococcus protective action in mice, the agglutinins for S pneumococci are specifically absorbable and are destroyed by heating the serum at 64° C. for 30 minutes.

It has also been observed that avirulent R pneumococci are agglutinated by hog serum. This agglutination is of typical R character. The agglutinated pneumococci

form a granular precipitate leaving the supernatant clear. The maximum R agglutinin titre is usually 1:250 to 1:500. So far no falling off of the R agglutinin titre has been seen even after the antipneumococcus protective action of the serum is lost.

Although sensitization of living pneumococci with hog serum does not alter their virulence, heat-killed pneumococci when sensitized by hog serum provoke a very meagre specific antibody response in rabbits.

With the concentrations used, no change in colony form has been brought about by growing either S or R, pneumococci in dilutions of hog serum. The R pneumococci cultured in presence of the serum grow in clumps.

X. Pathogenesis of Experimental Intradermal Pneumococcus Infection. (Dr. Goodner). The intradermal pneumococcus infection in rabbits offers an experimental disease which bears many analogies to lobar pneumonia. The general nature of this symptom-complex has been studied for a number of years and is now sufficiently well understood to serve as a basic method for more specific projects.

With Dr. Rhoads the histological pathogenesis of this lesion has been studied and found to be comparable to that of lobar pneumonia in man. The findings are described in detail in another section. The outstanding feature of the early lesion is the edema fluid; its accumulation occurs before any change in the cellular picture, and in the pro-

gressing or moving lesion its passage through the tissue precedes any other sign of reaction between tissue and micro-organism. It seems probable that the advancing fluid carries with it the first infecting organisms and consequently inoculates all tissues which it reaches. The resultant infection appears to be due, not to an active invasion, but rather to a progressive passive inoculation.

Edema fluid has been removed from the developing lesion and its properties studied *in vitro*. If obtained free from blood and tissue elements such fluid does not clot even after long standing. That this property is not due to a deficiency of fibrinogen is shown by the fact that a firm clot promptly appears on the addition of thromboplastin. Evidence points to a high antithrombic content since the edema fluid has the property of retarding the clotting of normal rabbit blood. The clotting time of the blood in these rabbits infected with pneumococci is prolonged, particularly in severe cases, but the effect is never as pronounced as in the exudative fluid where the concentration of the antithrombic factor appears to be much greater. The nature and source of this antithrombic substance are now being investigated.

A study has been made of the factors which have to do with the development, movement, final localization, and extent of the dermal lesion in the rabbit. The direction of movement is determined by gravity and is independent of the

distribution and course of blood vessels and lymphatics. The lesion seems always to seek to reach the more directly dependent area. The direction of movement may be modified by changing the position of the animal or by arranging artificial guiding structures such as scars. If the skin of the abdomen is the final site of localization there is a massive collection of edema fluid. Such edematous lesions are not obtained in areas where the skin is tense, such as over an extremity. Drainage distance largely determines the volume of edema fluid accumulating in a given area.

Numerous factors modify the development and rate of movement. Perhaps the most striking of those yet observed is the accelerating or synergistic co-infection with B. influenzae. This seems of particular significance since some form of the Pfeiffer bacillus is found in a large percentage of sputa from cases of lobar pneumonia. If B. influenzae and pneumococci (Type I) are injected together intradermally the effect is a marked acceleration in the development and movement of the lesion. The lag period of the pneumococcic infection is almost entirely eliminated. Other studies on the associative relationships of these organisms are now in progress.

I. Intradermal Infection with a Rabbit Virulent Type III Pneumococcus. (Dr. Goodner). The character of the intradermal infection with a rabbit virulent strain of Type III pneumococcus has been studied and found to follow essen-

tially the same course as with Type I, although the disease is much more severe and the fatality rate higher. Cases terminated more often by lysis than by crisis. This is possibly explained by the low grade active immunity which follows artificial immunization or recovery from the disease. Recovered animals show but slight resistance to reinfection even at short intervals. The immunity acquired from a course of the disease is of sufficient quantitative character to prevent death after reinfection but not sufficient to prevent a severe infection.

The use of Type III antiserum (by ourself and by others) in treating experimental infections has yielded poor results. A selected serum was concentrated by our method and although high agglutinin and precipitin titers were obtained, this concentrate fails completely to protect mice. It has, however, a favorable influence in the rabbit infection, although even in large amounts it seems incapable of promptly terminating the disease.

The enzyme which specifically decomposes the capsular polysaccharide of Type III pneumococcus is being used in treating this experimental disease. Preliminary experiments have given encouraging results but many factors are as yet not sufficiently understood to warrant final conclusions. The enzyme preparations as obtained at this time possess a primary toxicity for normal rabbits and are particularly toxic for infected animals. With certain preparations which

showed the lower range of toxicity the therapeutic effect was marked. The blood stream became free of organisms and after an initial rise the temperature dropped to normal levels. Repeated injections of the enzyme are necessary to prevent relapses.

XI. Study of the Transformation of the Specific Types of Pneumococcus. (Dr. Alloway). The experiments carried out by Griffith in 1928 for the first time brought convincing evidence that specific types of Pneumococcus are not absolutely fixed but may, under special conditions of growth, be transformed one into another. It had been known previously that type-specific pneumococci may be degraded into the so-called common R forms, but it was generally believed that, if, these forms ever again reverted to type, they always reverted to that type from which they were originally derived.

Griffith brought about the transformation of types by inoculating mice subcutaneously with small quantities of living R organisms together with large quantities of heat-killed S cells of a type other than that from the R strain originally came. Under these conditions, the R forms resumed the specific properties of the particular S type used in the heated inoculum. In other words, the process of reversion may now be specifically oriented at will, depending upon the type of Pneumococcus used in the reaction-mixtures.

Dawson in 1930, working in this laboratory, confirmed Griffith's results and later defined the cultural

conditions under which transformations similar to those originally induced in the animal body may now be brought about in the culture tube. Pneumococci which have assumed the specific characters of the new type *in vivo*, show no tendency to revert spontaneously to the original type. With the acquisition of a new specificity, the R forms also regain the property of virulence, a quality usually not restored by mere cultivation on artificial media. At present further studies are in progress with the hope of finding out the basic factors responsible for these remarkable changes in biological specificity. Attempts are being made to extract from type specific pneumococci, the substance, or substances, responsible for the activation of the R forms. Since the type-specificity of pneumococci is dependent upon the presence of the capsular polysaccharide, this substance in purified form was used instead of the whole cells. The failure to induce the change under these conditions indicates that the specific polysaccharide, in a chemically purified state, does not, by itself, function as the activating substance. Reference has already been made in this report, to a method of extracting the capsular polysaccharide in a form in which it still retains its antigenic property. Since acid extraction with heat has been found to remove from the cells this otherwise easily dissociated antigen, the same method was used in attempts to extract in active form the bacterial substance which is concerned in the process of transformation.

Extracts prepared in this way, from which formed cells were removed by prolonged centrifugation, have proved active in causing R pneumococci derived from Type II to assume the specific characters of the Type III S cells from which the extract was made. However, when the same extract was passed through a Berkefield filter it lost its activity and was no longer effective in bringing about the change. Although too little is known of the biologically active substances which appear to be specific activators of the R forms of pneumococci, the possibility exists that they may function as a co-zymase or activator of a zymase which exists in an inactive state in all R cells. This hypothesis furnishes at least a working basis and seems the more likely since it is now apparent that every R cell has potentially the capacity to synthesize any one of the type-specific capsular polysaccharides, which one it happens to elaborate being dependent upon a specific stimulus, or activator.

Various methods of extraction are being tried with the hope of obtaining the active substance from the type-specific cells. From such extracts it may then be possible to isolate the active principle and thus to obtain a more definite knowledge of its chemical and biological properties and its relation to the mechanism of this important phenomenon.

XII. Significance of Oxidation-Reduction Processes in Bacterial Growth. (Dr. Dubos). When Pneumococcus is seeded into plain broth, it is necessary to use a fairly large

inoculation in order to insure the initiation of growth. The work of previous years had established that this "bacteriostatic" action of the broth, as expressed by the lack of growth of very small inocula, can be corrected by a variety of treatments which have in common the development of reducing conditions in the medium. It has now been shown that the bacteriostatic substance is present in the peptone used in the preparation of the broth in which this substance usually exists in an oxidized form. It appears furthermore that this bacteriostatic substance can be reversibly oxidized and reduced and that the bacteriostatic properties are exhibited only by the oxidized form, and not at all by the reduced form. In this respect, it behaves like methylene blue, gallocyanine and the various indophenols, dyes which have been shown previously to be bacteriostatic only in the oxidized form.

Different brands of commercial peptones contain varying amounts of bacteriostatic substance. This substance, although its nature is not known, can be titrated by means of reducing substances, in particular the reduced thio-acids. The principle of the titration is to determine what amount of the reduced substance is necessary to correct the toxicity of a certain amount of the bacteriostatic material. Such titrations have revealed that the bacteriostatic action can be recognized with concentrations corresponding to as little as 10^{-5} molar concentration of thioglycollic acid.

These concentrations are of the order met with in catalytic reactions and one may wonder whether the bacteriostatic substance of the peptone does not act as anticatalyst in some phase of the metabolism of the cell.

It was of obvious interest to attempt to obtain peptone preparations free of this bacteriostatic action. This has been achieved by two completely different methods.

A. Purification of a brand of commercial peptone.

It has been found possible to remove 90-95 per cent of the bacteriostatic fraction of Fairchild peptone by precipitation with concentrated hydrochloric acid. The bacteriostatic substance thus precipitated appears as a pigment which can be reversibly oxidized and reduced. In the course of this process, the color of the material changes from green to brown; the bacteriostatic action is associated with the green (oxidized) form.

As mentioned previously, the acid precipitation removes only 90-95 per cent of the bacteriostatic material. Another bacteriostatic fraction is associated with the acetone soluble component of the peptone. A purified peptone was prepared by removal of these two fractions. With this purified preparation, a medium could be prepared in which a positive growth of Pneumococcus was obtained, in the absence of blood or any reducing substance, with an inoculum as small as .000,000,1 cc. of culture.

B. Preparation of peptones from pure proteins.

There are indications that the bacteriostatic substance is derived from some constituents of the animal tissues used in the preparation of the peptones, and it was thought that peptones prepared from purified proteins would be devoid of bacteriostatic substance.

In fact, peptones prepared by peptic or tryptic digestion of casein have justified this assumption. These casein digests, when used for the preparation of "plain broth" give media in which the growth of Pneumococcus can be initiated with very small inocula; it has also been observed that the quantity of growth, as measured by the number of cells or amount of bacterial protein synthetized, is much higher with these casein digests than with any of the ordinary peptones which have been tested. Work is in progress establishing the value of these peptones for the growth of other Gram positive cocci, of B. granulosis, of the anaerobic filter passing group, and of strict anaerobes. It may be mentioned that Dr. A. R. Dochez has used these preparations in experiments on the cultivation of virus of the common cold *in vitro*.

Equally satisfactory results, although not so complete, have been obtained with peptones prepared from other purified proteins (fibrin, globin).

XIII. B i b l i o g r a p h y.

Avery, O.T., and Dubos, Rene: The specific action of a bacterial enzyme on pneumococci of Type III. Science, 1930, 72, 151.

Avery, O.T., and Dubos, Rene: The protective action of a specific enzyme on Pneumococcus Type III infection in mice. (In manuscript).

Babers, F.H., and Goebel, W.F.: The molecular size of the Type III specific polysaccharide of Pneumococcus. J. Biol. Chem., lxxxix, 1930, 387-394.

Dubos, Rene: The bacteriostatic action of certain components of commercial peptones as affected by conditions of oxidation and reduction. J. Exper. Med., 1930, 52, 331-345.

Dubos, Rene; and Avery, O.T.: The decomposition of the capsular polysaccharide of Type III Pneumococcus by a bacterial enzyme. (In manuscript).

Francis, T.,Jr., and Tillett, W.S.: Cutaneous reactions in Pneumonia. The development of antibodies following the intradermal injection of type specific polysaccharide, J. Exper. Med., 1930, 52, 573-585.

Goebel, W.F.: The preparation of the type-specific polysaccharides of Pneumococcus. J. Biol. Chem., 1930, lxxxix, 395-398.

Goodner, K., and Rhoads, C.P.: Factors involved in the development and localization of the dermal pneumococcic lesion in the rabbit. (In manuscript).

Julianelle, L.A.: The distribution of Friedländer's bacilli of different types, J. Exper. Med., 1930, 52, 539.

Rhoads, C.P., and Goodner, K.: On the pathogenesis of experimental dermal pneumococcus infection in the rabbit. (In manuscript).

Stillman, E.G.: The effect of the route of immunization on the immunity response to Pneumococcus Type I. J. Exper. Med., 1930, 51, 721-727.

Stillman, E.G.: Susceptibility of rabbits to infection by the inhalation of Type II pneumococci, J. Exper. Med., 1930, 52, 215-224.

Stillman, E.G.: Development of agglutinins and protective antibodies in rabbits, after inhalation of Type II pneumococci, J. Exper. Med., 1930, 52, 225.

Stillman, E.G.: Duration of demonstrable antibodies in serum of rabbits immunized with pneumococci Type I, (In manuscript).

Tillett, W.S., and Francis, T.,Jr.: Serological reactions in pneumonia with a non-protein somatic fraction of Pneumococcus. J. Exper. Med., 1930, 53, 561-571, and J. Clin. Invest., 1930, 9, 11.

Tillett, W.S., Goebel, W.F., and Avery, O.T.: Chemical and immunological properties of a species-specific carbohydrate of pneumococci. J. Exper. Med., 1930, 52, 895-900.