

Report of Dr. Avery (assisted by Drs. Adams, Beeson, Cattaneo,
Daddi, Dublin, Dubos, Goebel, Goodner, Heggie, Hoagland, Hotchkiss,
MacLeod, Reeves and Stillman).

Treatment of pneumonia with antipneumococcal rabbit serum (MacLeod, Beeson, Dublin, and Heggie). One hundred patients suffering from pneumonia due to pneumococci comprising 12 different types, have been treated in the Hospital with unconcentrated antipneumococcal rabbit serum since this therapeutic agent was first used three years ago. The total mortality was 11 per cent, but if pneumonia due to *Pneumococcus* Type III is excluded, the mortality is only 5.4 per cent of 74 cases in which the infection was caused by other types of pneumococcus.

Unconcentrated antipneumococcal rabbit serum is a very satisfactory and effective therapeutic agent except in the cases of Type III pneumococcal pneumonia. 26 patients who suffered from Type III pneumonia were treated with serum, and of these 7 died. Of the patients with Type III pneumonia who died only three could be considered as having received adequate dosage of serum by our present standards, but the fact that these patients died despite intensive treatment, indicates that specific antiserum alone is not an entirely adequate therapeutic agent in Type III pneumonia.

The patients who died, other than those in the Type III group, numbered only 4. In two of these empyema was present associated with severe bacteremia; one patient had meningitis, and the fourth patient was an elderly man with Type II pneumonia who had involvement of four pulmonary lobes when admitted to hospital.

During the past 2 years an attempt has been made in each case to administer the required amount of antibody within as short a period as possible. This has resulted in more rapid defervescence in the patients so

treated, and is a more advantageous method of treatment than that of giving the serum in divided doses over a 12 to 24 hour period. 18 patients with Type I pneumonia were given the required amount of serum within a 2 hour period. In these patients, the time required for defervescence, as calculated from the time serum treatment was begun, was only 9.3 hours. This is less than half the time required for defervescence in Type I patients who were treated with concentrated horse serum in divided doses (22.6 hours).

The administration of serum has been greatly facilitated by the use of the skin test with the homologous type specific polysaccharides. The test has been used as a guide to dosage of both horse and rabbit antipneumococcal serum in patients with pneumonia caused by Pneumococcus Types I, II, III, V, VII, VIII. In 77 per cent of the 104 patients upon whom skin tests were done, the reaction was found to be entirely satisfactory in determining when a sufficient amount of serum had been given. 12.5 per cent of the patients showed a positive skin reaction before the administration of specific antiserum and at a time when the disease was advancing. In this group the test could not be used as a guide to therapy. Patients who die of the infection (10 per cent of this group) do not develop a positive skin reaction despite the presence of specific antibody in their circulating blood. In such individuals the skin test cannot be used as a guide to serum dosage, but is a valuable prognostic aid.

The skin test is of greatest value in patients who show a negative reaction before serum administration, and in whom serum is effective in initiating recovery, for on the appearance of a positive test, serum can be safely discontinued.

Treatment of pneumococcal infections with sulfapyridine. 2-(para aminobenzenesulfonamido)-pyridine or sulfapyridine has been advocated recently as a therapeutic agent in pneumococcal infections. Preliminary observations

indicated a definite protective action against intraperitoneal infections of mice with pneumococci Types I, II, III, V, VII and VIII, and a curative effect in patients suffering from pneumococcal infections. Consequently, the study of the action of this new sulfanilamide derivative was undertaken both in the laboratory and in the clinic patients with pneumonia.

Effect *in vitro* of sulfapyridine on the Pneumococcus (MacLeod and Dadd)

Sulfapyridine exerts a definite bacteriostatic effect on the Pneumococcus *in vitro* in high dilutions. The lag phase is prolonged and growth is less profuse than in the absence of the drug. Higher concentrations of drug may inhibit growth entirely. However, if transferred repeatedly in serum broth containing sulfapyridine, Pneumococcus Type I will tolerate gradually increasing concentrations of the drug until finally it is able to grow in concentrations which inhibit entirely the growth of organisms not so accustomed.

The sulfapyridine-fast Pneumococcus is encapsulated, and retains its virulence for the mouse. Indeed, sulfapyridine in amounts sufficient to protect mice against infection with Type I pneumococci fails to protect mice infected with the drug-fast strain derived from the parent culture. Preliminary observations indicate that the type specific serological and antigenic properties of the drug-fast organisms are not altered. The Neufeld reaction is positive, indicating that the function of capsule formation is not impaired and Type I serum protects mice infected with the strain resistant to the action of the drug. Investigation of the biochemical activity of this drug-fast strain is being undertaken.

Effect of sulfapyridine on pneumococcal infections of mice. The effect of sulfapyridine on the outcome of infection induced in mice with Pneumococcus Types I, II, and III differs markedly in each instance.

Mice infected intraperitoneally with 1,000,000 lethal doses of Pneumococcus Type I respond well to sulfapyridine administered by stomach tube.

Practically all of the treated mice survive infection if the administration of the drug is continued over a two day period. The mice which survive are found to be immune to re-infection with Pneumococcus Type I. With the same infecting dose of Pneumococcus Type II, even though treatment is prolonged for 4 days, approximately only 60 per cent of the mice will survive. In the case of Type III Pneumococcus, the survival rate is very low even though treatment is carried out for as long as seven days.

If normal mice are immunized intraperitoneally with a single injection of heat-killed pneumococci of these three types, and infected four days later with the homologous organism the survival rate is approximately the same as in the above experiments where sulfapyridine was used. In other words, the survival rate in mice infected with Pneumococcus Types I, II and III and treated with sulfapyridine appears to be related to known differences in the antigenic potency of these three types of pneumococcus: the better the immune response to the infecting inoculum, the higher the survival rate.

In the case of infection of mice with Type III, synergism exists between the action of specific antiserum and sulfapyridine. Amounts of serum and drug, which by themselves fail to protect mice, exert a high degree of protective action when used in combination.

Treatment of pneumonia with sulfapyridine (MacLeod, Beeson, Dublin and Heggie). On the basis of experimental observations the treatment of pneumococcal pneumonia with sulfapyridine has been undertaken. With all infections other than those due to Pneumococcus Type III, treatment has been carried out with the drug alone, if this was possible, but serum has been used as an adjunct when the outcome of the infection has appeared doubtful. However, in the case of Type III pneumonia, treatment with the drug alone has not been considered to be justified, so that in these infections a combination of sulfapyridine and Type III unconcentrated antipneumococcal rabbit serum has

been used.

A beneficial action of the drug on the outcome of pneumococcal pneumonia seems undoubted. Crisis within 24 hours from the beginning of drug administration has occurred in the majority of patients even though treated at all stages of the disease and despite the presence of bacteremia.

In the case of Type III pneumonia, synergism appears to exist between the action of sulfapyridine and specific antiserum. In three instances sulfapyridine alone has failed to induce sterilization of the blood of the invading organisms, but this has occurred promptly with subsequent serum treatment. However, the amount of serum used has been considerably less than has been necessary previously in treating patients with Type III pneumonia.

Nausea and vomiting have been an almost invariable accompaniment of sulfapyridine administration, and have been severe enough to necessitate the parenteral administration of fluid in about one half of the patients so affected.

Hemolytic anemia has occurred in three patients, all of whom subsequently recovered. This phase of the toxicity of sulfapyridine has been investigated in collaboration with Dr. C. P. Rhoad's department. In the cases studied, the occurrence of hemolysis, as measured by the excretion of urobilinogen, has not occurred unless a total dosage of 20 gms. of sulfapyridine has been given.

Acute azotemia has occurred in three patients, in one of whom acute hemorrhagic Bright's disease has supervened. The problem of the effect of sulfapyridine on renal physiology is being investigated in collaboration with Dr. D. D. Van Slyke's department.

The primary toxicity of the compound may limit seriously its use unless means are found of reducing the noxious effects.

The conversion of creatine into creatinine by a bacterial enzyme

(Dubos). A bacterial enzyme capable of oxidizing creatinine to urea, and the use of this enzyme as a specific reagent for the quantitative analysis of creatinine, have been described in earlier reports. It has now been found that the same bacterial species which produces this oxidizing enzyme also produces another enzyme which converts creatine into creatinine. This reaction offers an opportunity for the study in vitro of (a) the enzymatic production of a biologically important cyclic compound from an aliphatic one, and (b) the enzymatic combination of an amino and a carboxyl group to form the important CO-NH linkage.

The new enzyme has been obtained in aqueous solution, free of the cellular structure. It exhibits a high degree of specificity for creatine, since it does not convert the closely related compound glycoxyamine into glycoxyamidine, nor does it change hydantoic acid into its anhydride, hydantoin.

The enzyme is not produced when the bacterial cells are grown in a medium which does not contain creatine or creatinine; it belongs, therefore, to the group of "adaptive enzymes" which are produced only in the presence of their specific substrate, or related compounds. The mechanism of the formation of adaptive enzymes has often been discussed in the past and it has been suggested in particular that the adaptation serves a useful purpose to the organism. The fact that creatinine - the end product of the reaction - is as effective as creatine in stimulating the formation of the enzyme under consideration is convincing evidence that the "adaptive" response cannot be explained on teleological grounds. It is apparent that the presence in the culture medium of some linkage or structure common to both creatine and creatinine is necessary and sufficient to cause the formation by the micro-organism of the enzyme which catalyses the reaction creatine - creatinine, irrespective of whether or not this reaction is of any use to the organism.

Properties of a nucleohistone antigen extracted from pneumococci

(Dubos). The preparation of a nucleohistone fraction, present in R and S pneumococci, has been described in the preceding report. The serum of rabbits immunized with this soluble fraction is capable of protecting mice against infection with virulent pneumococci. Although the protective action is not entirely specific since it is effective against several types of pneumococci, it is always of a much higher degree against the type of pneumococcus from which the antigen has been prepared.

In recent experiments, the nucleohistone antigen has been prepared from R pneumococci derived from Type II. The sera of rabbits immunized with this antigen sometimes protect mice against small doses of Type I pneumococci; the same sera, on the other hand, always protect against large doses of Type II organisms. Interestingly enough, these sera fail to agglutinate suspensions of pneumococci (living or heat-killed) of any type tested, nor do they precipitate soluble fractions extracted from them, not even the nucleohistone used as antigen in the preparation of the immune sera. The sera do not appear to exert any bacteriostatic or bactericidal effect on pneumococci and in fact, the mechanism of the protection which they induce is still unexplained.

It is usually considered that, among proteins, histones and protamines are peculiar in not being antigenic. It is true that the *Pneumococcus* nucleohistone considered in the present report fails to produce any antibody detectable by the precipitin reaction, but the protective effect exhibited by the sera of rabbits immunized with this substance seems to indicate that the latter possesses antigenic activity. It appears possible, therefore, that like the pneumococcus nucleohistones other histones and protamines may in reality act as antigens, but that the antibodies to which they give rise are not detectable by the classical precipitin reaction.

Bactericidal effect of an extract of a soil bacillus (Dubos). It is well known that most living cells are resistant to the action of common enzymes; this is true in particular of pathogenic microorganisms which are not affected by any of the known tissue enzymes.

During the past few years we have made a number of attempts to discover whether certain microbial species are endowed with enzymes which permit them to cause the lysis of the living cells of Gram positive cocci. To this end, suspensions of living pneumococci, streptococci and staphylococci were repeatedly added to different samples of soil over a period of three years in the hope that a soil flora would develop, capable of specifically attacking the living cells of the same bacterial species repeatedly added as specific substrate. From these soils, an unidentified spore bearing bacillus has recently been isolated which is capable of causing the lysis of living Gram positive cocci; the lysis takes place through the agency of a soluble substance which is produced by the soil bacillus in the course of its growth. The soluble factor has been separated from the bacterial cells and can be purified by precipitating it at its isoelectric point (pH 4.6).

The purified bacterial extract exerts a bactericidal effect on all Gram positive cocci so far tested and prevents their growth in laboratory media. The phenomenon of lysis, however, is not observed with all the species which are killed by the extract; many species of streptococci, for instance, fail to undergo lysis in the presence of amounts of extract far in excess of the amount required to destroy their viability. In fact it appears that, in the cases where it is observed, lysis is only a secondary phenomenon due to the action of the autolytic enzymes of the affected cells following the primary injury inflicted upon the living bacteria. Although it is not yet possible to form an opinion as to the nature of this primary

injury, it has been found that the glucose dehydrogenase of the bacterial cell is inactivated by the extract before any morphological alteration can be recognized.

The extract is effective in vivo as well as in vitro and protects mice against infection with virulent pneumococci and streptococci. For instance, a daily dose of 1 mgm. administered by the intraabdominal route on three consecutive days protects mice against 10 million fatal doses of pneumococci. The extract also exerts a curative effect when administered several hours after infection. The degree of protection afforded, and the minimal amount of extract required, are the same irrespective of the type of pneumococcus used as infective agents; experiments have so far been conducted with pneumococci of Types I, II, III, V, and VIII.

Tests carried out in collaboration with Dr. R. C. Lancefield have shown that the protective effect of the extract against virulent hemolytic streptococci is of the same order as that against pneumococci; experiments have been instituted with 10 strains of group A and 2 strains of group C hemolytic streptococcus, all with successful results.

It is worth emphasizing again that the amounts of extract required for protection against infection are the same irrespective of the types of pneumococci or hemolytic streptococci used as infective agents. It is clear, therefore, that the extract exerts its effect by altering some structure, or interfering with some function which is common to all Gram positive cocci and which is essential for maintaining their metabolic activity.

Finally it may be mentioned that, in spite of many attempts, it has been impossible to observe any effect of the extract against any of the Gram negative bacilli so far tested.

On the nature and behavior of antibodies (Goodner). It now appears that several different serum proteins may have the properties of antibodies; indeed it seems certain that in some immune sera one antigen may react with more than one variety of antibody protein. Moreover, within any one class of serum proteins, there appear to be antibodies having varying affinities for a single antigen. Precise chemical analyses have shown that, with some antigen-antibody systems, the addition of increasing amounts of antigen gives results in terms of antibody precipitated which do not form a curve but which give a figure of ascending straight lines. With the simplest possible system only one ascending phase (facet) is obtained. Other systems have given examples of two, three and four ascending phases. Each of these phases of constant antigen-antibody ratios appears to be related to the participation of a single regional or spacial configuration of the antigen, each of these being dominated by a chemical combining group. The inference would be that a single antigen forms a series of antibodies of sharp specificity and that these antibodies react with the antigen in a perfectly definite order, this order being determined by Mass Action equilibria. The antibody which forms the least soluble compound with the antigen is first precipitated, the next least soluble compound second, etc. For any one system the ratios for any one phase are definite but are altered by alteration of the system as by the removal of certain of these highly specific antibodies. In other words, the equilibria are altered depending upon the other reactive components of the system.

If large excesses of antigen are added to antisera the well known phenomenon of prozone is obtained. In this the antigen-antibody combination is perfectly soluble. Space limitation does not permit the explanation of this matter but it may be said that from the chemical point of view those antibodies which are first precipitated by small amounts of antigen are the

first to form soluble compounds on the addition of excessive amounts of antigen. The order of precipitation and of inhibition of precipitation are the same.

In simpler systems the region of maximum precipitation is characterized by an absolute plateau. In more complex systems the antibody first precipitated may be involved in an inhibitory phase even before the last reactive component has been precipitated and the configuration may appear to be that of a curve due to the general complexity.

General significance of these findings:

1. A single antigen does not give rise to a single antibody as has long been assumed, but engenders, rather, a series of antibodies each sharply specific for a particular chemical region of the antigen.

2. Each combination of a single antibody with its related region of the antigen follows the simple laws of classical chemistry.

3. On the basis of these findings it is possible to offer simple and clear explanations for such immunological puzzles as: Cross reactions, the Danysz phenomenon, the prozone, and many others.

A specific protein found in the blood during infection (Avery, MacLeod and Hotchkiss). As previously reported the blood serum of a patient acutely ill with pneumococcus pneumonia or with an infection due to organisms other than pneumococci yields a precipitate when a dilute solution of the pneumococcus "C" polysaccharide is added to it. The serum of normal individuals fails to react when similarly tested. Moreover, no other polysaccharide has as yet been found which yields a precipitate with sera known to contain the "C"-reactive substance. Patients, whose serum gives a positive precipitation test, develop a local and typical skin reaction at the site of injection of 0.1 mg. of the test carbohydrate. Both the precipitation test and the skin

reaction are positive during the acute phase of the infection and become negative when the patient recovers. The early appearance and the subsequent disappearance of the reactive substance in the blood, as determined by the results of these tests, parallel closely the clinical course of the infection; both reactions remaining positive with continued activity of lesion, and becoming negative with termination of the infectious process.

In collaboration with Dr. Hotcakiss considerable progress has been made in the isolation and identification of the "C"-reactive substance. It has been recovered in purified form from the blood of fatal cases of pneumococcus pneumonia, and has been shown to be a protein possessing certain chemical and immunological properties which distinguish it sharply from the normal serum proteins. This protein has been separated by fractional precipitation of the serum with sodium or ammonium sulphate; the active protein flocculates from the albumin fraction on dialysis against tap water. The separation thus effected has been found to depend on two factors, namely, the presence of small amounts of calcium in tap water and the occurrence of serum lipids in the albumin fraction.

The role of calcium ions in relation to certain properties of this protein is of special interest; the fact that the active protein is sensitive to calcium has been utilized in the process of isolation and purification, and in the study of its reactivity with the "C" polysaccharide. Reference was made in the preceding report to the fact that the precipitation reaction with the test carbohydrate is conditioned by the presence of calcium in the reacting system; for example, no visible precipitate forms when "C" is added to reactive serum from which the calcium has first been removed. However, the re-addition of a mere trace of calcium chloride suffices to cause precipitation. Moreover, the "C" precipitate from reactive serum is dissolved

when the calcium ions are removed by adding oxalate or citrate. It is now known that in the absence of other electrolytes, calcium chloride at the concentration five ten-thousandths molar, is sufficient to precipitate the protein. For this reason the active protein separates out of solution when the albumin fraction of "pathological" serum is dialyzed against ordinary tap water. Dialysis against distilled water at the same pH does not result in precipitation. However, with the concentration of electrolytes in physiological saline solutions, a considerably higher concentration of calcium chloride, about two-hundredths molar, is required to bring the protein out of solution.

The reactivity of the protein with calcium under these conditions appears to be due to the presence of a lipid substance. When lipids are removed from the serum by extraction at low temperatures with alcohol and ether, the active protein can no longer be precipitated from the mixture by calcium ions. The precipitability by calcium can be restored by adding the albumin fraction of a serum containing lipids, such as normal human serum or normal rabbit serum. The precipitation reaction with "C" polysaccharide, however, is not affected by extracting the lipids from the serum or by defatting the protein after isolation from reactive serum.

It is evident that the interplay of calcium and a lipid or lipids present in reactive serum determines the unique insolubility of the active protein by virtue of which it can be separated in relatively pure form from the accompanying inert serum albumin. While calcium is essential in the precipitation reaction with the "C" polysaccharide, the lipid material is not necessary. Since both calcium and the lipid material have been demonstrated in normal human serum neither of these components can be considered as being a specific result of the infection, whereas the presence of the

"C"-reactive protein bears a direct relationship to the infectious process. Moreover, normal serum does not react with the "C" polysaccharide, nor has it been possible by the methods described to demonstrate in normal blood a substance having the unique chemical and immunological properties which characterize the active protein found in the serum of patients during the acute stage of bacterial infection.

The active protein isolated from pathological serum is relatively resistant to the action of such proteolytic enzymes as crystalline trypsin, chymotrypsin and crude commercial preparations of the pancreatic enzyme. However, the crude enzyme preparation slowly destroys the activity of the protein.

Immunological properties. The "C"-reactive protein in purified form is markedly antigenic giving rise in rabbits to antibodies which are specifically directed against this particular protein. Rabbits immunized with the "C" precipitate containing the active protein produce antibodies which specifically react with "pathological" sera and with solutions of the active protein prepared from them. Absorption of rabbit antiserum with normal human serum removes all precipitins for the latter but leaves undiminished in titre the precipitating antibodies reactive with patients' serum and with the specific protein isolated therefrom.

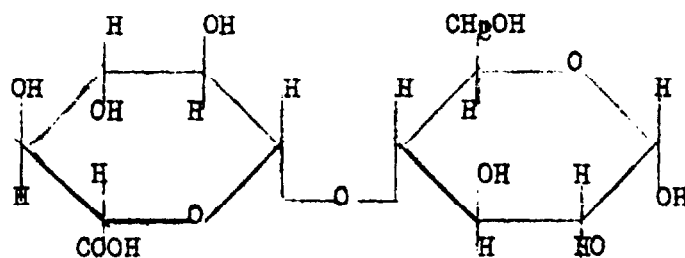
Through the courtesy of Dr. Goodner, complement fixation tests have been carried out using antisera of rabbits immunized with the proteins of normal human serum or with the active protein from patient serum. Rabbit antiserum to the normal proteins of human blood failed to fix complement in the presence of the specific protein isolated from patients's serum. On the other hand, rabbit antiserum to the specific protein from "pathological" serum fixed complement with the same antigen in dilutions as high as 1:500,000.

By precipitin and complement fixation reactions it is possible, therefore, to differentiate specifically the normal serum proteins from the "C"-

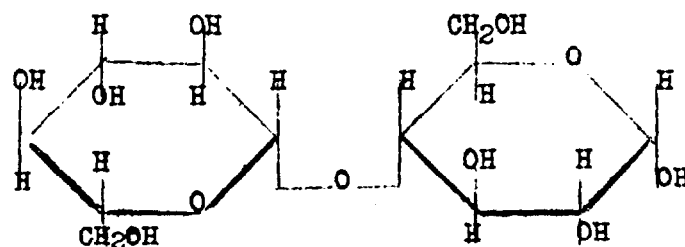
reactive protein which circulates in the blood during the acute phase of bacterial infection.

Immuno-chemistry of carbohydrates of synthetic and bacterial origin (Goebel, Beeson and Adams). During the past year considerable progress has been made in the major problem under investigation in this laboratory, namely, the rôle of carbohydrates in infection and resistance. This problem has been approached in two distinct ways. First, artificial antigens have been prepared containing simple carbohydrates of known chemical structure. The immunological properties of these antigens have been studied and correlated with changes in chemical constitution. These studies have revealed that the stereochemical configuration of simple hexoses, the configuration and position of intramolecular linkages of disaccharides, as well as the conversion of the primary alcohol group of mono- and disaccharides to the carboxyl group are all important determinants in orienting the specificities of simple carbohydrates. A comprehensive understanding of the sharply defined specificities exhibited by encapsulated microorganisms cannot be attained until the exact chemical structures of the capsular polysaccharides themselves have been ascertained. Our second approach, therefore, has been a detailed study of chemical constitution of the specific polysaccharides of certain types of the pneumococci.

Synthetic antigens: A. Cellobiuronic acid. In the previous report the preparation and serological properties of two artificial antigens, one containing the disaccharide cellobiose, and the other cellobiuronic acid, the building stone of the Type III pneumococcus polysaccharide, were described. Cellobiose and cellobiuronic acid differ only in the nature of the group occupying the 12th position as can be seen from the following graphic formulae.



Cellobiuronic Acid

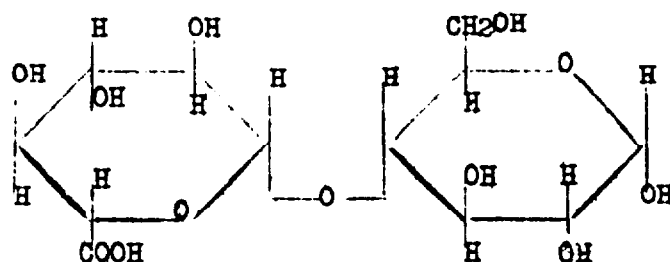


Cellobiose

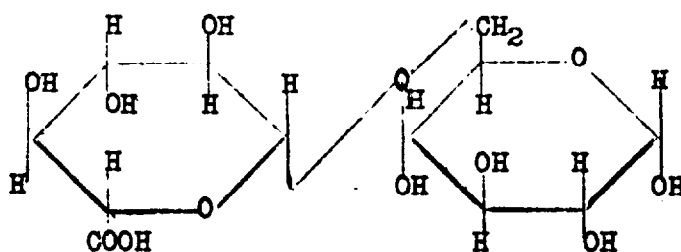
In the disaccharide cellobiose this grouping is a primary alcohol (CH_2OH) and in the aldobionic acid a carboxyl group (COOH) occupies this position. This slight difference in chemical constitution suffices however, to confer upon each antigen entirely different immunological properties. The serum of rabbits immunized with an antigen containing the azobenzyl glycoside of cellobiuronic acid agglutinates in high dilution Type III pneumococci and causes typical swelling of the capsule. This serum also precipitates Type III pneumococcus specific polysaccharide and confers passive protection on mice against infection with multiple lethal doses of virulent Type II, III and VIII organisms. Following immunization with this antigen, the rabbits themselves are actively resistant to dermal infection with Type III pneumococci. On the other hand, the sera of rabbits immunized with the antigen containing the corresponding glycoside of cellobiose exhibit none of these phenomena.

B. Gentiobiuronic acid. Gentiobiuronic acid, first prepared synthetically in this laboratory, is an isomer of cellobiuronic acid. Both

uronic acids are constituted from a molecule of glucuronic acid linked in glycosidic union to a molecule of glucose, the configuration of the intermolecular glycosidic linkage is in each instance the same. The two aldobionic acids differ only in the position of glycosidic linkage. In cellobiuronic acid this linkage is through the 4th carbon atom of the glucose molecule, and in gentiobiuronic acid the linkage is through the 6th carbon atom. The structural relationships of these two aldobionic acids is represented by the following graphic formulae.



Cellobiuronic acid



Gentiobiuronic acid

During the past year the synthesis of gentiobiuronic acid from 1:2:3:4 tetracetyl glucose and α -bromotriacetyl glucuronic acid methyl ester has been perfected. The corresponding heptaacetyl gentiobiuronic acid methyl ester has been converted to α -bromohexacetyl derivative and from this various gentiobiuronic acid glycosides have been synthesized, among them the p-aminobenzyl gentiobiuronide. The latter derivative has been coupled with serum globulin to yield an artificial antigen containing the azobenzyl glycoside of the uronic acid.

This artificial antigen, containing a carbohydrate radical of synthetic origin, exhibits certain of the serological properties of pneumococcus polysaccharides. It precipitates in high dilutions in antipneumococcal horse sera of Types II, III, V, and VIII. A corresponding antigen containing the disaccharide, gentiobiose, has likewise been prepared. When rabbits are immunized with these antigens, the antibodies evoked are in each instance specific and selectively differentiate between the disaccharide and the uronic acid derivative. Furthermore, the antibodies to the gentiobiuronic acid antigen are serologically different and distinct from those obtained by immunization with the isomer, cellobiuronic acid. Moreover, unlike the latter immune serum, gentiobiuronic acid antiserum contains no agglutinins, precipitins, or protective antibodies for Type III pneumococci. The striking differences in protective action against Type III infection of the antisera to these two closely related biuronides demonstrates that the aldobionuronic acid constituent of the antigen must possess an exact stereochemical pattern in order to evoke antibacterial immunity.

Structure of Type III pneumococcus polysaccharide (Goebel, Adams and Reeves). Because of the importance of carbohydrates in determining the type specificity of encapsulated pathogens an extensive investigation is being carried out on the chemical constitution of certain of the bacterial specific polysaccharides. We have chosen the specific polysaccharide of Type III Pneumococcus for investigation.

The capsular polysaccharide of Type III Pneumococcus is constituted from units of cellobiuronic acid (4- β -glucuronosido-glucose) joined in glycosidic union to form a macromolecule. We are at present engaged in the problem of determining the position of the glycosidic union between the aldobionuronic acid units in the intact polysaccharide molecule.

On reducing the esterified acid groups of the fully methylated capsular polysaccharide by catalytic hydrogenation, a new polysaccharide is obtained in which the acid groups of the aldobionic acid residues are replaced by primary alcohol groups. On acid hydrolysis the reduced polysaccharide yields the known 2:3:6 trimethyl glucose (derived from the methylated glucose constituent of the original aldobionic acid unit) and an unknown dimethyl glucose (obtained from the reduced glucuronic acid portion of the cellobiuronic acid). We are at present attempting a rigorous synthetic proof of the structure of this derivative, which we have reason to believe is 2:4 dimethyl glucose. When this synthesis is completed we shall know the entire chemical structure and hence shall be in a position to define the exact chemical basis for the unique specificity exhibited by the Type III polysaccharide.

The capsular polysaccharide of Type XIV Pneumococcus and the relationship to the group specific substances of human blood (Goebel, Beeson and Hoagland). It has been found that Type XIV antipneumococcus horse serum contains agglutinins for human erythrocytes of the different blood groups. Since this phenomenon is peculiar to Type XIV antiserum, it was thought that an investigation of the properties of the capsular polysaccharide of Type XIV Pneumococcus might reveal points of similarity with the blood group specific substances, and at the same time shed light upon the mechanism whereby antipneumococcal serum of this particular type agglutinates human erythrocytes.

The capsular polysaccharide has, therefore, been isolated from Type XIV pneumococci. The products of hydrolysis of the carbohydrate have been investigated. The polysaccharide has been found to be constituted from one molecule of acetyl glucoseamine and three molecules of galactose linked in glycosidic union to form a non-diffusible macromolecule. This polysac-

charide is unique in that it is the first type specific carbohydrate of bacterial origin we have isolated which does not contain uronic acids within the molecule. The substance resembles both in its chemical and immunological properties the blood group A specific substance isolated and identified during the course of the past year from different brands of commercial peptone.

Type XIV antipneumococcus horse serum precipitates both the homologous polysaccharide and the blood group A specific substance in high dilution. When the antiserum is absorbed with the bacterial polysaccharide not only are all homologous precipitins and agglutinins removed, but agglutination of human erythrocytes and precipitation of the blood group specific A substance no longer occurs with the absorbed serum. Absorption of the serum with the A substance or with erythrocytes of A, B, and O groups removes the agglutinins for all blood groups, but not the antibodies which react with Type XIV pneumococci or with the capsular polysaccharide derived from them.

Others have reported that administration of Type XIV antipneumococcus horse serum to pneumonia patients has in some instances been followed by hemoglobinuria and death. These untoward reactions can now be attributed to a similarity in structure of the Type XIV specific polysaccharide and the blood group substances as reflected in the immune serum.

The synthesis of aldobionides and the relationship between the molecular rotation of derivatives of acetylated aldoses and uronic acids (Goebel and Reeves). In continuing the chemical and immunological investigations on artificial antigens it has become desirable to prepare azo proteins containing aldobionic acids. A general method for the synthesis of the glycosides of aldobionic acids has therefore been devised. This method consists in converting the aldobionic acid to the methyl ester which is in turn acetylated to yield isomeric heptaacetates. These substances are then

converted to the α -bromo derivative, from which any desired glycoside can be obtained by the conventional method. In the course of our studies on uronic acids we have been struck by the fact that a correlation seems to exist between the values for the molecular rotation of certain mono- and disaccharides and their corresponding uronic acid derivatives. A comparison has therefore been made of the molecular rotations of various acetylated derivatives of these saccharides in which an acetyl, methoxyl, or halogen group has been substituted on the first or aldehydic carbon atom. It has been observed that in all instances the values for the molecular rotation of each saccharide and the corresponding uronic acid derivative differs by a small and approximately constant amount. It is apparent, therefore, that a change in molecular rotation, the value of which approximates a constant, accompanies the conversion of the terminal acetylated primary alcohol group (CH_2OH) to the carboxymethyl group (COOMe). This relationship can be expressed by the equation - $[\text{M}]_{\text{D}_1} - [\text{M}]_{\text{D}_2} = \text{K}$ where $[\text{M}]_{\text{D}_1}$ is the molecular rotation of the hexose uronic or aldobionic acid derivative and $[\text{M}]_{\text{D}_2}$ the molecular rotation of the hexose or disaccharide derivative. We consider this newly discovered rule to be of value in prognosticating the configuration of the derivatives of hexose-uronic and aldobionic acids.

Differences in virulence of various types of pneumococci for mice

(Stillman). Most experimental work of late years has been carried out with strains of pneumococci selected because of their pathogenicity for certain laboratory animals. Many of these strains have been under artificial cultivation for long periods of time, and most of them have been repeatedly passed through mice, so that their virulence for this particular species of animals has been selectively increased. Since pneumococci of the different specific types are associated with infections of varying severity in man it was of interest to determine whether strains of this organism freshly

isolated from human sources varied in their initial virulence for mice.

Two different methods have been used for determining the primary infectivity of pneumococci in mice. In all instances the cultures have been tested as soon as possible after direct isolation from patients, in order to minimize possible changes resulting from artificial cultivation. Studies have been made of the virulence of these microorganisms as measured by their capacity to invade the animal's tissues when the living bacteria are inhaled and thus implanted on the uninjured respiratory mucosa of mice. The results obtained by the inhalation method have been compared with those following the injection of these same organisms through punctured tissues into the peritoneal cavity. The former method more closely resembles the natural route by which these organisms gain entrance to the respiratory tract of man and serves as a measure of their ability to penetrate the defensive tissues of the normal mucous membranes.

From this study it is apparent that there exist great differences in the virulence of freshly isolated strains of pneumococci when tested by direct inhalation method and by interperitoneal injection. Although the number of strains of pneumococci and the number of mice exposed to each strain are too few to draw any definite deductions, the results indicate that Type I pneumococci have a relatively low virulence for mice irrespective of the route by which they gain entrance to the animal body. Type II organisms have higher virulence as judged by the number of fatal infections induced in mice following inhalation or interperitoneal injection. Under the same experimental conditions, Type III and Type VIII organisms both possess a much higher degree of virulence than do the strains of Type I and Type II which have been tested. The incidence of fatal septicemia is highest in mice exposed to infection with Type III pneumococci.

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