

Dr. Avery's report on the Chemo-Immunology of Pneumococcus.

An important advance in the study of infectious diseases came with the discovery that microorganisms, although simple in form and with but few points of structural differentiation, nevertheless possess differences in biological specificity as diverse and as sharply defined as are those characteristic of the more complex forms of life. These differences are not detectable by the ordinary methods of cultivation; indeed, bacteria of the same species, which look alike under the microscope and which grow alike on artificial media, have by the more delicate methods of immunology been found to possess differential specificity as distinctive as that exhibited by members of unrelated species. Knowledge of this immunological specificity among bacteria is becoming of increased importance for the proper understanding of control of infectious diseases. Such study is not only necessary in the elucidation of the biological relationships existing between varieties of the same species of bacterium, but is essential to the working out of epidemiological problems and to the development of methods useful in the control of infectious diseases by means of specific therapeutic and prophylactic measures. Leaving out of consideration the promising but difficult field of chemotherapy, the problems of specific cure and prevention of infection lie in the attempt to interpret and imitate by artificial immunization certain protective processes of nature which constitute what we call immunity. In order to imitate successfully the natural processes involved in spontaneous recovery from disease it is necessary to know the nature of the underlying immunity developed and clearly exhibited in the specific reactions between the infectious agent and the body tissues of the host. The specific nature of these biological processes is

advantageously studied by means of the so-called immunity reactions which serve as a measure of the capacity of the animal organism to produce protective substances or antibodies against infection and which afford a means of studying the interaction between specific antibodies and infectious microorganisms when both are brought together in the animal body or in the test tube.

Investigations on the specificity of these reactions have added much knowledge to the many and diverse problems of infectious disease. It will suffice, as an illustration of the progress that has directly grown out of immunological investigation, to recall the advance in our knowledge of diphtheria; we now possess not only a specific curative agent in the form of antitoxin, but a sensitive method based upon a specific reaction for detecting susceptible and immune individuals, and finally a specific means of artificially immunizing the susceptibles against infection by treatment with balanced mixtures of toxin and antitoxin.

The study, however, is one of varying complexity, the methods suitable in one instance fail utterly when applied to another type of infectious disease. For this reason it at once becomes obvious that the hope of further advance resides in an attempt to relate these specific differences in biological behavior to fundamental differences in the chemical constitution and intimate properties of the microorganisms themselves.

With this end in view, those of us on the hospital staff engaged in the clinical investigation of acute respiratory disease are at present seeking to acquire a more intimate knowledge of the immunological and chemical constitution of the pneumococcus, - the most

frequent and one of the most deadly microbial incitants of pneumonia in man. It seems desirable at this time to attempt a brief summary of such progress as has been made in this aspect of a problem, upon the ultimate solution of which rests the hope and power of man's defense against this fatal disease.

It will not be necessary to refer in detail to the earlier studies carried out in the wards and laboratories of this hospital except in so far as the facts they have revealed are related to and form the basis of the more recent work. You are already familiar with the initial impetus that was given to the study of pneumonia through the working out of the biological classification of pneumococci, which made possible the recognition sharply defined and specific types within this previously confused species. You will recall that by the application of this method it was possible to determine the frequency of occurrence of these specific types in pneumonia and the relative differences in the severity of mortality of the infections they produce; that a study of the presence of the classifiable types of pneumococci in the mouth secretions of healthy individuals proved the dissemination of the disease-producing varieties by healthy carriers and convalescents, and led to a new interpretation of the epidemiology of the disease; and finally that the knowledge of type specificity among pneumococci has furnished a rational basis for the development of an immune serum which in the treatment of pneumonia due to Type I infection has proved of distinct therapeutic value.

Helpful as these contributions have been to clinical medicine, they still leave unsolved many perplexing questions. If pneumococci are so widely disseminated why do some people escape

while others contract pneumonia; if the specific relationships between pneumococci are now known, why can't a specific means of preventive inoculation be devised and generally applied; if a curative serum has been perfected against one of these disease-producing types why can't an equally potent serum be produced against the others? These and a host of other searching queries immediately suggest themselves. The answers to these questions lie in the fact long since recognized that the processes of infection and immunity are much "more subtle than the resultant of a mere bringing together of pathogenic microbe and susceptible host"; they involve an intricate series of delicately balanced reactions in which the biological properties of the pathogenic microbe and the specific response of the animal tissues determine the outcome. The mere accession of even a virulent type of pneumococcus does not necessarily lead to pneumonia unless contributing factors other than those relating to the microorganisms are present. From clinical observation of the spontaneous disease in man, and from the study of infection experimentally produced in animals some knowledge has been gained of these contributing factors which quite apart from the bacterial themselves, tend to break down the normal defensive mechanism of the respiratory tissues. Among these extraneous factors, the most important in man appear to be the ordinary upper respiratory infections, such as common colds, but these do not lead to pneumonia, at least by the highly parasitic pneumococci unless the individual has acquired by direct or indirect contact one of the virulent types of pneumococcus. The disease then may be considered to occur only when both the contributory causes and the bacterial incitant become operative together.

The problems relating to the possibility of preventive

inoculation and the perfecting of curative sera for infections due to pneumococci of Types II and III are in essence the objectives of our present endeavors. Progress in this direction has been impeded by the lack of a more precise knowledge of the essential differences which have always been suspected but only recently demonstrated to exist between the actual chemical constitution of pneumococci belonging to the three specific types. What these chemical differences are, upon what chemical constituent of the cell this biological specificity depends, and how intimately this specific substance is concerned in determining the invasive properties of the bacterium and the immunity response of the body tissues, are questions which in part at least have yielded to recent investigation.

Pneumococcus is a unicellular organism which under well defined conditions of growth is surrounded by an envelope of material known as the cell capsule. The development of this capsular layer is particularly pronounced in pneumococci which possess the capacity to grow and multiply in the animal body. It is also known, that during the processes of growth these cells elaborate a substance which dissolves out into the culture fluids in which the organisms are growing, and that this substance retains in this soluble form all the type specificity of the intact cell from which it arises. Not only is this soluble specific substance found in suitable cultures, but its presence is readily demonstrable both in the body fluids of animal experimentally infected and in the blood and urine of individuals suffering from pneumococcus pneumonia. The function of elaborating this specific material is likewise most highly developed in the most virulent types of pneumococci, and there are grounds for the belief that the capsule of these cells is

largely composed of this soluble specific substance. Thus, there is disposed peripherally about the cell a capsular layer which reacts in a specific manner with the serum of animals which have been previously immunized by the repeated injection of the dead bodies of pneumococci of the same type. The reaction which occurs under these circumstances is remarkably specific; for instance, pneumococci of Type I or the soluble specific substance derived from organisms of this type react only in Type I antipneumococcus serum and similarly the bacteria or the specific substances of Type II and Type III pneumococci react only in the corresponding antiserum of the homologous type. These specific reactions form the basis of the original classification. But, the actual isolation of the important specific substance in a purified state, the determination of its chemical constitution and the study of its relationship to the immunological properties of the cell as a whole, are problems in the solution of which considerable progress has only recently been made. Stripped of technical detail the status of our present knowledge of these matters is briefly as follows: The specific substance of pneumococcus when chemically isolated from the cell in a purified form has been found to belong to the great groups of sugar-like substances, namely the carbohydrates. No matter from what type of pneumococcus these specific substances are isolated they all possess in common the chemical properties of complex sugars. But interestingly enough, the sugar derived from each of the three specific types of pneumococcus is chemically distinct, each possessing unique properties which serve sharply to differentiate it from the others. Moreover, solutions of these bacterial sugars in their chemically purified state exhibit the same type specific reactions in immune serum as do the bacterial bodies from which

they are derived. Some idea of how remarkably sensitive and specifically reactive these bacterial sugars are may be judged from the fact that by the use of an appropriate serum their presence may be detected in dilutions as high as 1 to 5,000,000. The fact that the particular constituent which determines the type specificity of pneumococcus is chemically a carbohydrate is an unexpected discovery, for, heretofore, all immunity processes have been considered to be purely protein-anti-protein reactions. Furthermore, the fact that these bacterial sugars are as chemically distinct one from the other as they are serologically specific for each type of pneumococcus is the most striking example of the intimate relationship between the chemical constitution and biological specificity of bacteria.

Another important and unusual property of these specific sugar derivatives is that sensitive as they are in reacting with serum antibodies, they are by themselves wholly devoid of the power to stimulate the formation of antibodies when they are injected into the animal body. In other words, separated from the cells of which they form a part these specific sugar substances still retain their original property of combining with antibodies but lose the power of invoking antibody response in animals. This latter property, their true antigenic function, they possess only in the form in which they exist bound in the cell. So long as they remain united in chemical combination with the other constituents of the cell they serve as extremely potent antigens in stimulating the tissues of the host to form immune protective substances. As soon as the cellular union is disrupted, however, they cease to function as true antigens and exhibit only the passive property of combining with and thus neutralizing the preformed antibodies. These

facts require special emphasis, for in the antibody-neutralizing property of these substances lies their vital significance in infection; and in the ease with which they lose their antibody-stimulating power when dissociated from the cell lies one of the greatest difficulties associated with the experimental production of antipneumococcus serum. The fact that this splitting of the specifically immunizing complex of pneumococci occurs so readily, particularly in the case of organisms of Type II and III and the fact that under these circumstances the stimulus to specific antibody production is lost so quickly in these two instances affords a possible explanation of the lack of success in obtaining an antiserum of high potency against these types. It seems not unlikely that the relative differences in the rate and degree of splitting of the specific antigens in the three types of pneumococcus are in each instance referable to known differences in the chemical structure of the specific sugar components. Antigenic stability, like specificity itself, then rests upon the chemical constitution of these unique and specific substances.

Some of the factors which bring about dissociation of the specific immunizing substance or type-specific antigen of the cell in vitro are already known and methods directed toward preventing their operation are being devised. Similarly the nature of the factors which make for dissociation of the antigen after infection into the animal body are being studied. In some way not yet fully understood, these latter factors appear to be related to what is commonly called natural immunity, for animals which are most resistant to pneumococcus infection are just those animals which have been found to possess the greatest capacity to split the antigen and consequently to yield the

least potent serum. This, then in a brief way, is the essence of our present knowledge of the so-called soluble specific substance of pneumococcus.

By suitable chemical methods we have also isolated from pneumococci another substance which forms the major part of the cell body namely, protein, which in chemical properties is like the nucleoprotein material of most cells. Unlike the type-specific sugar derivatives, the protein fraction possesses no differential specificity from an immunological point of view; it is, so far as we can determine chemically and antigenically the same for all types and varieties of pneumococci. Unlike the bacterial sugars the pneumococcus protein when isolated from the cell body still possesses the power of stimulating antiprotein antibodies when injected into animals. However, the kind of antibody to which it gives rise in the serum of treated animals reacts with the protein isolated from all types of pneumococci. In other words, pneumococcus protein is common to the species and possesses none of the type differential qualities which distinguish the specific carbohydrate substances of the cells. Moreover, the antibodies to which this protein gives rise on immunization while reacting with the protein of all pneumococci have no effect on solutions of the isolated bacterial sugars, or on the intact cells which are layered about with a capsule containing these type specific substances. But if the pneumococcus is first stripped of its capsule, which you will recall is largely composed of specific carbohydrate, then the naked protein body of the cell is exposed to and acted upon by serum containing these antiprotein antibodies. Here then, is another important fact in the biology of pneumococcus. Among other constituents the bacterial cell is composed of two

chemically distinct substances, each of which function quite differently in antipneumococcus immunity; one the carbohydrate of the capsule which is type specific, the other the protein of the cell body which is only species specific.

Finally, reference must be made to the capacity for variation which pneumococci exhibit under certain conditions of growth. Although fixity of type relationships within the species is remarkably constant, variations may be experimentally induced and may even occur spontaneously. The first changes recognized and studied were in the direction of degradation. Type specific, encapsulated, virulent pneumococci when subjected to an unfavorable cultural environment more or less quickly undergo a change which is associated with the loss of a function rather than with the acquisition of new characters. Pneumococci in which this change has occurred, while still maintaining the purely vegetative functions of growth and reproduction are no longer virulent, type specific or encapsulated. They have lost the function of elaborating the type specific carbohydrate of which the capsule is composed. Associated with lack of this function is the loss of virulence, for pneumococci which suffered this change are no longer able to invade the animal body. These non-virulent, unencapsulated cells, like the naked cells from which the type specific capsular material has been stripped by chemical means exhibit only the protein - antiprotein reactions common to the species.

Whether these degraded forms of pneumococci, having once lost all the specific characters which originally distinguished their parasitic antecedents, can ever again revert to the biological type from which they sprang, is a question concerning which there has been

considerable doubt and controversy. By special methods of cultivation outside the animal body we have recently demonstrated that under certain conditions these degraded cells may be caused to revert and that the process of reversion is accompanied by the restoration of capsular development, type specificity and animal virulence. Further studies are necessary to determine the nature of the stimulus which induces these reversible reactions.