streptococci, a system that is being adopted in all parts of the world. The second has been the guiding motive in investigating sensitive tissues and cells; and tests have been carried out both in the intact animal and in tissue cultures. A point has now been reached where it is hoped that it will be possible to investigate the relationships of two variables - bacterial hypersensitivity and true immunity, in experimental streptococcal diseases and in rheumatic fever and other human infections.

Pneumonia

Dr. Cole, Dr. Avery and their Associates.

Dr. Cole, Dr. Avery and their associates have devoted a large amount of time to the clinical study and treatment of pneumonia patients in the wards of the hospital, and to investigations in the laboratories on biological and chemical problems relating to pneumococcus infection and immunity. The records of the clinical studies provide a wealth of source material for studying the natural history of the disease and form a most valuable asset, which is constantly being employed in the formulation of new work and in checking the results of special investigations. The two contributions by Drs. Goodner and Horsfall and by Dr. Dubos, which are given in detail later, emphasize the mutual advantages of laboratory activities carried on in close contact with clinical work, for, in both instances the results of laboratory experimentation have found direct and practical application in the clinic.

In addition to the general remarks concerning activities in the wards and laboratories of the hospital it seems advisable to present a more detailed account of some work that has been done, (1) by Dr. Goodner and Dr. Horsfall, associates of Dr. Avery, (2) by Dr. Dubos also an associate of Dr. Avery, and (3) by Dr. Smadel and Dr. Farr, associates of Dr. Swift and Dr. Van Slyke respectively.

Type specific antipneumococcus rabbit serum in the treatment of lobar pneumonia. The past quarter of a century has seen enormous advances in the understanding of lobar pneumonia. It is now recognized that lobar pneumonia is not one disease, but a long series of maladies presenting similar symptoms caused by a number of different, although related, bacteria. Much is now known of these bacteria, the pneumococci, and of the way in which extraordinarily severe infections are produced by them. Much also has been learned of methods of specific therapy, for it was soon recognized that each of these forms of pneumonia must be treated with a special agent, each agent being specifically directed against the particular variety of pneumococcus causing the infection. The development of specific serum therapy by Cole and his associates forms one of the most important chapters in the history of this hospital.

Specific serum therapy is based essentially on the following observation: If patients spontaneously recover from lobar pneumonia, it is almost invariably found that at the time of recovery there appear in the patients' blood new substances called antibodies, which are capable of reacting with the particular microorganism that caused the infection. It was reasoned from this observation that recovery might be hastened and the disease aborted if some of these antibodies could be supplied from another source; many patients die before they can develop their own immune substances. In order to test this possibility, horses were immunized with dead pneumococci, since these serve as well as living, and the blood serum of these horses was administered to patients. This form of therapy, if administered early in the course of the disease, proved remarkably successful in infections caused by four of the 32 varieties of the pneumococcus. The mortality rates were definitely lowered in infection with these four types, but even here the treatment left much to be desired.

As a natural part in the development of specific therapeutic measures came the question of whether or not the horse was the most desirable source of the antibodies. It has only recently been recognized that the immune serum from one animal species may possess properties remarkably dissimilar to those of the immune serum from another species. Most of these differences reside in the antibodies themselves. One may take as an example the immune serum from the horse and the rabbit. Both possess antibodies which have the common property of reacting with the pneumococcus and its chemical constituents. Beyond that, all similarity is lost. The number of differences now recognized is over thirty. In considering these differences in antibodies it became apparent, in theory at least, that the immune rabbit serum should serve as a much more efficient therapeutic agent than is immune horse serum in treating human lobar pneumonia. This subject is somewhat technical and it is unnecessary that it be discussed at length in this place, but as a simple example the implications of one of the differences may be mentioned. The antibody in immune rabbit serum has a diameter one-third the length of that of the antibody of immune horse serum. Because of this smaller size, it was reasoned, the rabbit antibodies might be better able to penetrate the tissues and reach the site of infection more readily than would the horse antibodies. Since pneumonic consolidation is primarily an extravascular lesion, as too are most of the various complications resulting from it, any agent which is to act locally on the lesion or its cause must do so after penetrating tissues.

These theoretical deductions were exceedingly interesting, but the effort to prove their validity by clinical demonstration has involved many problems. For example, it has long been known that rabbits are readily immunized by means of injections of pneumococci, but the use of rabbit serum for the treatment of human lobar pneumonia demanded an intensive investigation. It might at first sight appear to be prohibitively expensive to use the serum

f small animals, but as the work progressed it became apparent that the im-
une rabbit serum could be produced at a comparatively low cost. This was an
important point, since the cost of concentrated horse serum has made the treat-
ent of lobar pneumonia somewhat impractical from an economic standpoint.

A second problem of considerable importance had to do with the toxicity of immune rabbit serum, for with the treatment of the first patient it was recognized that this material in a raw or untreated form could produce the most alarming chill reactions. Considerable progress has been made in dealing with this problem; methods have been devised for reducing or eliminating this toxic quality, and methods of testing for its presence have been made available.

The results of the clinical application of type specific immune rabbit serum in the treatment of lobar pneumonia have justified the hopes gained from the theoretical studies which preceded its adoption. Sera have been produced against nine of the more common varieties of pneumococci and these sera have been used in the treatment of about 70 patients suffering with lobar pneumonia. The results have been good. Among more than fifty patients suffering with lobar pneumonias due to pneumococcus Types I, II, V, VI, VII, VIII, XIV, XVIII, there has been but one death and this occurred in a patient five weeks convalescent from pneumonia. In untreated patients with similar type distribution the death rate would have amounted to about 34 per cent. With lobar pneumonia due to Pneumococcus Type III the immune rabbit serum has not proven to be uniformly successful, but with certain improvements in the quality of the serum and in the methods of administration the therapeutic possibilities with this type have become very promising.

In evaluating the advantages of antipneumococcus rabbit serum as a therapeutic agent one must consider not only the mortality rates but what actually happens in individual cases. With antipneumococcus horse serum great

stress was placed on the possibility of lowering the mortality rates. With this new serum, so successful has been its application that stress has come to be placed on the matter of prompt recovery. In the last several cases treated with immune rabbit serum in this hospital, the average time from the first injection of serum until the crisis was less than nine hours. In many patients normal temperature, pulse, and respiration were regained in as short a time as five hours after serum was administered. To one familiar with the results obtained with immune horse serum these results are striking.

Another result which had been anticipated from theoretical grounds is that the number of complications has been very low in this series of treated patients. Empyema, the accumulation of pus in the pleural cavity, has occurred only once and this was in an individual with defective circulation. It has been possible to demonstrate that the specific antibodies of the immune rabbit serum penetrate into the infected fluids which lead to this complication and bring about sterilization.

An acquaintance with the details of this subject tempts the conclusion that considerable advance has been made in the treatment of an infectious disease, lobar pneumonia, which is one of the greatest causes of death.

The Production of Specific Bacterial Enzymes

Organic compounds exhibit a remarkable specificity in their physiological action. Unfortunately the chemical methods available for the identification and quantitative analysis of these compounds are on the contrary very non-specific. Many of these methods utilize reagents developed for certain chemical groups of general occurrence and which may be common to many different substances otherwise unrelated. The lack of specific chemical methods has therefore rendered more difficult the study of physiologically active substances, especially when they are present in small amounts in complex tis-

sues and biological fluids.

It is known that the lower forms of life (bacteria and molds in particular) manifest a great specificity in their ability to decompose different organic substances. Since practically all organic matter in nature eventually undergoes microbial decomposition, and in view of the fact that microorganisms act through the agency of their enzymes, one may infer that with proper techniques it should be possible to find in the microbial world a number of enzymes adapted to the decomposition of most types of organic compounds.

Two entirely different problems have offered the opportunity of demonstrating that bacterial enzymes specifically directed against given substances can indeed be produced and that these enzymes can be used to great advantage as reagents in biological studies.

A few years ago, an enzyme capable of decomposing the capsular polysaccharide of Type III pneumococcus was extracted from the cells of an unrelated bacterial species. The enzyme is very specific in its action against the polysaccharide, and was used in an analysis of the role played by this cell constituent in determining the immunological specificity and in conditioning the virulence of encapsulated pneumococci.

More recently, in the course of studies on renal function, it became necessary to develop a method for the quantitative estimation of the very small amounts of creatinine present in blood. The identification and analysis of creatinine in biological fluids have chiefly depended on colorimetric methods which are so non-specific, that many authors deny the very presence of creatinine in the circulating blood. In an attempt to solve this problem, two bacterial enzymes have been prepared which exhibit a remarkable specificity for creatinine, decomposing it completely after a short incubation, but failing to attack other substances which are closely related to it and which give the same color reactions. With the help of these enzymes, it

has been demonstrated that creatinine is indeed present in blood plasma and in the erythrocytes. Quantitative studies of the amounts of creatinine present in the blood and urine of normal individuals and nephritic patients have also led to the conclusion that other substances (probably phenols), often confused with creatinine because they give the same test with the non-specific colorimetric method so far employed, accumulate in the blood during uremia and fail to appear in the urine. This observation indicates a marked impairment in the renal excretion of toxic compounds and may afford a test of the efficiency of kidney function.

The two examples presented above illustrate the possible applications of specific bacterial enzymes in the study of biological problems. The principles of the methods used in the preparation of these enzymes will now be considered, with special reference to creatinine.

No animal or plant tissue is known to decompose creatinine. It is obvious, however, that creatinine does not accumulate in nature and therefore must undergo decomposition probably through the agency of microorganisms. To test this assumption, creatinine was added to samples of a number of different experimental conditions obtained by varying systematically the factors of temperature, humidity, aeration, reaction, etc. It was found that creatinine was rapidly decomposed in certain samples of soil incubated aerobically at 37°C and at neutral and acid reactions. In order to favor the growth of the microbial species specifically adapted to the decomposition of creatinine, the mixtures in which this material had undergone decomposition were inoculated into a variety of liquid media in which creatinine was the sole organic compound present. Again, creatinine was decomposed in some of these preparations and by repeated transfers in the same media, four active bacterial species were eventually isolated in pure culture. Only two of these cultures (NC and HR) have been carefully studied.

It was found that these two cultures grow well on common peptone media; but, whereas the creatinine splitting enzyme was readily formed when the organisms were compelled to use creatinine in the course of their growth, only little or no specific enzyme appeared under other conditions. A similar situation had already been observed in the case of the bacterium which decomposes the capsular polysaccharide of Type III Pneumococcus. These so-called "adaptive enzymes", which are formed only as a response to the presence of a definite substrate in the medium, appear to exhibit a remarkable degree of specificity in their action. This is illustrated in the few following examples. Creatinine is 3-Methyl glycocyamidine. The mere presence of an additional methyl or acetyl group in the molecule completely inhibits both enzyme NC and HR. The removal of the methyl group in position 3 (leaving glycocyamidine) decreases the activity of both enzymes by 90 per cent. The shift of the methyl group from position 3 to position 5 retards considerably the action of enzyme NC and completely inhibits enzyme HR. Finally, substances which give the same color reactions as creatinine but are otherwise unrelated in structure are not attacked at all by either enzyme.

The production of a definite enzyme by bacteria as a response to the presence of the corresponding substrate in the medium is a striking example of specific adaptation, and may eventually be used in an analysis of this phenomenon. But in the meantime it may also offer to the biochemist a method for the preparation of reagents specifically adapted to the study of many types of organic compounds.

Nephritis

Dr. Van Slyke and Associates.

The work of Dr. Van Slyke and his associates is centered chiefly on problems arising from a study of nephritis. These involve: (1) Clinical work