

sick and will not be subjected to certain dangers associated with primary vaccination with calf lymph virus, but will obtain a solid and lasting immunity to smallpox. Furthermore, it is possible that a cultured virus may be developed which will be suitable for intradermal use and will not require prompt dermal revaccination with a potent calf lymph vaccine to produce an enduring immunity. These matters are for future investigation,

Although the final objective desired has not been attained, much progress toward it has been made. During this progression the first cultured virus for use in human beings was prepared and many observations on the cultivation of viruses have been made. These are valuable to colleagues working in similar fields, notably those striving to perfect vaccines for the prevention of yellow fever and influenza.

PNEUMONIA

Dr. Avery and Associates.

Serum therapy in lobar pneumonia has been fostered in our Hospital for many years. This type of treatment had reached a high state of perfection when eighteen months ago chemotherapy of the disease suddenly appeared upon the scene. The ease with which the latter form of therapy can be administered and its relative inexpensiveness necessitated a study of its merits by us, particularly in view of the fact that our figures on serum therapy are the best in existence. During the past year such a study has been conducted and will be continued during the coming year. In addition, the work of Dr. Dubos has disclosed a very interesting and potent substance obtained from a soil bacillus, which may act as a key to further studies on chemotherapy.

The production of antibacterial substances by soil microorganisms.

Organic matter does not accumulate in nature; the dead bodies of plants and

animals that fall upon the earth and all the waste materials which find their way into sewage water, eventually become the prey of countless species of microorganisms that break them down to the most simple forms of matter found in the mineral world. The complex process which changes a decaying block of wood, or a dead animal, into carbon dioxide, water, ammonia and simple mineral salts cannot be performed by any one single microbial species. Countless different kinds of molds and bacteria in turn attack the many different organic molecules of which living tissues are composed. Furthermore, each one of these microbes performs only a limited, well-defined task. It is, therefore, assumed that - given enough time, patience and skill - bacteriologists will eventually discover in nature microorganisms selectively adapted to the decomposition of almost any type of organic substance.

During the past few years this principle has found its application in the isolation from soil of bacteria that carry out reactions of interest to the biochemist and the bacteriologist. Two examples will serve to illustrate the method and its possibilities.

I. The decomposition of the capsular polysaccharides of pneumococcus by bacterial enzymes. Virulent pneumococci are surrounded by a capsule consisting of complex carbohydrates which protects these bacteria against the normal defense mechanisms of the animal tissues. The capsular substances, although fairly simple in structure, cannot be decomposed by any of the cells or fluids of the animal tissues. When the same capsular substances are added to soil, however, they are attacked by certain soil bacteria which use them as a source of food; the same bacteria isolated in pure culture and removed from the soil can also decompose the capsular substances in the test tube.

It has been possible to obtain in solution the enzymes - the digestive juices - by means of which the soil bacteria dissolve the capsular substances. The same enzymes, separated from the bodies of the bacteria that produce them, are capable, both in the test tube and in the animal, of destroying the pneumococcus capsules, and as a matter of fact can be used to protect animals against infection with virulent pneumococci.

This example illustrates the different steps by which one can obtain from the soil selective microbial agents capable of attacking given substances. The substance to be decomposed is added to soil in order to stimulate the multiplication of soil microorganisms capable of using it as a source of food. The active microbes are then separated from the soil and isolated in pure culture. Finally the agent by means of which they perform the reaction - a soluble enzyme for instance - is separated from the microbial bodies, purified, and its activity is tested.

II. Preparation and properties of a bacterial agent which kills Gram-positive microorganisms. The soil bacteria which have just been mentioned do not kill the pneumococcus cell; they do not affect its essential life functions, but only dissolve its capsular substance. It appeared possible that there might exist in nature microorganisms capable of attacking, not only well defined chemical substances, but also the living cells of other, unrelated microbial species. In the first place attempts were made to discover soil microorganisms antagonistic to the disease-producing Gram-positive cocci (pneumococci, streptococci, staphylococci), a group of bacteria in which workers in our Hospital have been interested for a long time.

Pneumococci, streptococci, staphylococci, although they differ with respect to their pathogenicity and cultural characteristics, have in common a number of properties that place them in one natural group. Their

similarity in structure is reflected in the fact that they can be stained by the Gram technique - a differential staining method widely used in bacteriology. In fact, all the microbial cells which take the Gram stain - the "Gram-positive bacteria" - can be clearly set apart from the Gram-negative organisms by a number of morphological, cultural and biochemical properties.

In order to find out whether there exist in soil any microorganisms capable of attacking the living cells of Gram-positive bacteria, suspensions of living pneumococci, streptococci, and staphylococci were repeatedly added to a sample of earth, in the hope that the continued presence of these foreign cells would stimulate the development of a soil population capable of living at their expense. This in fact did happen, and it was possible to isolate from the sample of soil, a sporulating bacillus capable of killing and destroying the living cells of Gram-positive bacteria.

It was soon observed, however, that the soil bacillus does not actually feed on the living Gram-positive cells. It first kills them by means of a powerful bacterial poison (a "bactericidal" substance) and then utilizes their dead bodies. The present report deals with the isolation and properties of the chemical substance, secreted by the soil bacillus, which causes the death of the pathogenic cocci. The antibacterial ("bactericidal") substance is abundantly produced when the soil bacillus is grown in a peptone solution. It can be separated from the peptone culture by extraction with alcohol, in which the substance is very soluble, followed by precipitation in aqueous salt solution, in which it is practically insoluble. The material which precipitates out of the salt solution is thus obtained free of the bacterial cells and carries all the bactericidal activity of the original culture. Minute amounts of this material are sufficient to kill in the test tube enormous numbers of Gram-positive bacteria, and to

protect mice against infection with virulent pneumococci and streptococci.

The material obtained by extraction with alcohol and precipitation in salt solution is not, however, a single substance. One can separate from it, by differential solubilities in alcohol-ether mixtures, two fractions which are very closely related in chemical structure, but which differ in some of their biological activities. One of these fractions has been obtained in crystalline form and will be referred to as the "crystalline" fraction. The other has not yet been crystallized, although it has been obtained in a state of purity. For reasons that will become obvious later it has been called the "protective" fraction.

Much is already known of the chemical composition of these two fractions. Suffice it to say that they possess the same essential molecular structure and that the protective fraction differs from the crystalline product by the possession of a simple chemical grouping, which makes up only a small fraction of the total molecule.

Both fractions exhibit in the test tube a remarkable bactericidal activity against Gram-positive microorganisms, e.g., 0.01 - 0.02 mg. of either substance is enough to kill 1,000,000,000 pneumococci or streptococci within 2 hours at 37°C. Staphylococci, Gram-positive bacilli, and even yeast cells are also killed by the same substances in the test tube, although the susceptibility varies from one microbial species to another.

Still smaller amounts of either fraction inhibit the growth of Gram-positive bacteria in nutrient broth. This is particularly striking in the case of pneumococcus which fails to grow in nutrient broth containing a dilution of 1/500,000,000 of the active substance.

It is not yet clear how the bactericidal substance exerts its killing action upon the susceptible cells; it is only known that, although

the affected cells retain unimpaired many of their biochemical activities, they lose the ability to oxidize certain essential foodstuffs and consequently die of starvation. In any case, it is important to emphasize that, whatever the nature of the mechanism or its action, the active substance has been found effective only against Gram-positive organisms, whereas the Gram-negative bacilli, for example, those of the colon-typhoid group, remain unaffected.

As stated above, both the crystalline and the protective fraction are effective in the test tube. The crystalline fraction, however, appears to be ineffective in the animal body and fails to protect mice against infection with virulent pneumococci and streptococci. On the contrary, one single dose of the protective substance injected into the abdominal cavity is sufficient to protect mice against 10,000 fatal doses of pneumococci or streptococci administered by the same route. The material has proven equally effective against infection with the five different types of pneumococci and the fourteen different types of hemolytic streptococci (group A and C) which have been tested so far. It is permissible to hope, therefore, that it will also prove effective against all virulent strains of these bacterial species, irrespective of type specificity.

When slightly larger doses, 0.002 - 0.005 mg., of the bactericidal agent are used and when the treatment is repeated on three consecutive days, it is possible to protect mice against 1,000,000 fatal doses of Type I or Type II pneumococcus. Furthermore, it is also possible to cure mice of a well established infection when treatment is delayed for several hours after injection of the infective organisms. Many mice treated two to six, or even seventeen, hours after infection with Type I or Type III pneumococcus were saved, although all the control untreated animals died between 30 and 44

hours after infection.

It must be pointed out that in all these experiments, both the infecting organisms and the bactericidal substance were injected by the intra-abdominal route. To date less success in treating animals by any other route has been attained; only a few of the infected animals were saved when the bactericidal substance was injected intravenously, subcutaneously or intramuscularly. Several possibilities may be invoked to account for this failure: a) The active substance when introduced in the general circulation may be eliminated so fast that it never reaches an effective concentration; b) it may be inactivated in some tissues, for instance by hydrolysis or conjugation; c) the active substance is known to be very insoluble in aqueous media and may, therefore, fail to diffuse and reach the different foci of infection.

Little is known as yet of the toxicity of the material; 0.1 to 0.2 mg. of the protective fraction injected by the intra-abdominal route will often kill mice within 48 hours. Smaller amounts, although they do not kill the animals, cause some loss of weight and degenerative changes of the different organs. Rabbits are also susceptible and are killed within 24 hours following the injection of 5-10 mg. of the material. It is evident, therefore, that the protective substance is a very potent toxin for mammalian species; it is also true, however, that so far no toxic effect has been recognized with the small amounts of substance which are sufficient to protect animals against infection. Finally it must be pointed out that the crystalline fraction which, although very bactericidal in the test tube, is inactive in the animal, also fails to cause any obvious toxic reaction in rabbits and mice.

The findings just reported have revealed the existence and to some extent the chemical nature of a new type of bactericidal agent which, al-

though extremely active against Gram-positive microorganisms, fails to attack Gram-negative bacilli. This selective affinity for a certain type of microbial cell may be of importance in deciding the value of a substance as a therapeutic agent. Any bactericidal agent to be effective in the body must attack selectively the microbial parasite, without injuring the cells of the host to be treated. It is encouraging to note, therefore, that the bactericidal agent described in the present report is not equally toxic for all types of cells, but on the contrary exhibits a selective affinity for a well defined group of microorganisms. In this respect it will become important to study the reasons that determine the specific affinity of the bactericidal substance for the Gram-positive cells. An understanding of this affinity may serve as a guide further to enhance the specificity of the agent and decrease at the same time its affinity for the tissue cells of the infected host; sharper specificity would probably decrease the toxicity for the mammalian tissue cells and increase its effectiveness against the microbial cells in the animal body.

It has already been pointed out that the toxicity and the insolubility of the material limit its effectiveness in the animal body; it is worth while considering, therefore, whether means can be found to overcome these limitations. It will be recalled that the crystalline fraction, although very active in the test tube, is neither active in the animal body, nor toxic for the mammalian organisms. On the other hand, the toxicity of the protective fraction, as well as its ability to protect animals against pneumococcus and streptococcus infections, is associated with the presence in the molecule of a minor chemical grouping not present in the crystalline fraction. It appears possible that, by replacing this differentiating chemical grouping by some other group properly selected, one may decrease the

toxicity of the natural bacterial product, while maintaining its protective value. It is also possible that by adding to the molecule a chemical group with strong affinity for water one may render the material more soluble in tissue fluids, and at the same time allow it to diffuse more rapidly through the organs.

So far, the experimental work has been concerned only with pneumococcus and streptococcus infections. It must be remembered, however, that the bactericidal agent is very effective against other types of Gram-positive organisms. It has been shown for instance that it kills very rapidly the cells of a Gram-positive spore-bearing aerobic bacillus. The anthrax bacillus belongs to this group, and it will be important to determine the effect of the bactericidal agent on this disease-producing microbe both in the test tube and in the animal body.

It has been suggested earlier in the report that there will probably be found in nature, in soil for instance, microorganisms capable of attacking the living cells of other, unrelated microbial species. From cultures of these soil organisms it should be possible to release free of the bacterial cells the agent by means of which they attack the susceptible species. In the present report there have been described the preparation and properties of such an agent which is active against Gram-positive microorganisms. It is permissible to hope that one will eventually discover soil organisms which attack other types of disease-producing microbes - the Gram-negative bacilli, the acid fast bacteria, etc. - and that the active substance by means of which they exert their antagonistic effect will be isolated. These agents may not themselves be effective in the animal body. An understanding of their chemical nature and of the mechanism of their action will undoubtedly, however, give the bacteriologist and the chemist new com-

pounds and new techniques for the development of chemotherapy on a rational basis.

CARDIOVASCULAR DISEASES

Dr. Cohn and Associates.

These workers are investigating diseases of the heart and blood vessels. In this group of maladies is the disorder called "essential hypertension", i.e., unexplained high blood pressure. From the work already conducted the investigators have been led to believe that in many cases of unexplained high blood pressure a reason for the disorder may be found. For example, quite a high percentage of their cases shows renal abnormalities, such as constricted renal blood vessels, constricted outlets of the kidney, or kidney stones. Having found in some of the patients a possible reason for the high blood pressure, they hope that by intensive examination of the remaining members of the group to uncover, in some of them at least, the cause of their trouble. In other words, these workers are of the opinion that the high blood pressure in patients with essential hypertension may be explained, and that the reason for it will not always be the same.

DISEASES OF THE BLOOD

Dr. Rhoads and Associates.

For a number of years Dr. Rhoads and his group have conducted clinical and experimental investigations on sprue, pernicious anemia, refractory anemias, leukemia, and a number of other diseases. During the course of their work, Dr. Rhoads came to believe that many of the maladies, although presenting entirely different clinical pictures, had nevertheless something in common, namely a conditioned deficiency. Indeed, he brought a certain amount of evidence that this idea deserves consideration, for many