THE PRINCIPLES OF SUBCUTANEOUS MEDICATION BY MEANS OF BACTERIA AND BACTERIAL PRODUCTS

MICHAEL I. REIFFEL, CHICAGO

That there existed small living organisms, too small to be seen by the unaided human eye, has been the thought of scientists since the dawn of history. Several philosophers of old were bold enough to state openly that such organisms existed, and few writers went so far as to frame their speculation on the subject which seemed like anticipations of future discovery. No one ever was able to see these minute organisms until Anton von Leeuwenhoek, the Dutch microscopist, a skilled lens grinder, completed his microscope, and spent several years examining a great variety of natural objects. In the course of his study he chanced to come upon the organisms now known as bacteria—(1683).

Leeuwenhoeck's observations remained isolated and without further improvement until 1786, in which year the Danish zoologist O. F. Muller clearly recognized the difficulty of studying such minute organisms. However, in spite of these obstacles that he encountered Muller succeeded in discovering many structural details of which his predecessors had been ignorant. Indeed, several kind of bacteria were so accurately described by him that they can be identified today as belonging to one or another of the chief group forms.

Another great advance was made by Ehrenberg, 1795 to 1876, who worked upon the animalcules found in infusions of hay and meat. The chief merit of Ehrenberg's work lay in the system that it introduced into the study of micro organisms. Ehrenberg was able to establish a number of different groups among the organisms now called bacteria and recognized fundamental differences between larger forms, such as screw or spiral shapes, rod shaped and spherical and several of the true protozoa with which they had up to this time been classed.

In 1796, Edward Jenner, an English physician and a coworker with Hunter and Harvey, discovered a new system for the treatment of variola which proved a great success. By

taking from an animal infected with variola some blood and injecting it into another animal of the same species, he found that it produced a diseased condition similar to that of the first animal, but in a less virulent form, and that it soon subsided, and that it further protected that animal from subsequent attacks.

On May 4th, 1796, Dr. Jenner injected into the arm of a boy a small quantity of lymph, obtained from a milk maid affected with variola, and found that this produced results similar to those obtained in his animal experiments; the disease was less virulent and the subject soon was protected from it. This was the first injection into man of a substance to protect him against the ravages of disease. Later in the same year he used the lymph of cows and called this liquid vaccine. With this liquid he protected the lives of thousands from the ravages of smallpox.

Van Helmont and Needham, after a number of years of study of these small organisms offered to the world the theory that these forms of life arose by spontaneous generation in such fluids as meat infusions and other organic solutions even after they had been boiled. This was contested by Spallanzani, who showed that when a meat infusion had been boiled three quarters of an hour and kept from contact with the air, the developments of micro organisms would not take place. It was then claimed by the adherents of the theory of spontaneous generation that the expulsion of air by boiling and the arrangements which prevented it from re-entering the vessels also prevented spontaneous generation. Schulze and Schwan then devised methods to permit the entrance of air after it had been heated in a glass tube or passed through sulphuric acid. Schwan also showed that certain poisonous chemicals when added to meat infusions prevented the development of micro organisms, and these chemicals we now call antiseptics. Schroeder allowed air to enter vessels containing boiled organic matters, through glass tubes, which had been plugged with cotton; no growth of organisms resulted. This is the method now used to keep pure cultures of bacteria from becoming contaminated.

Pasteur in 1860 showed that a short boiling of an infusion of organic matter was not sufficient to kill all micro organisms and that some could withstand the temperature of boiling water

for several hours. This was substantiated by F. Cohn and Robert Koch, who also showed these resistant forms of micro organisms to be the spores of bacteria. In 1878 Robert Koch completed his brilliant experiments in the making of solid culture media. This enabled the scientific world to recover micro organisms from diseased animals by growing them on this media from the blood or secretions obtained from that animal, and to successfully study their morphology. Koch demonstrated that the changes which took place upon the contact of bacteria and media were those of decomposition, caused by bacteria.

Pasteur, while engaged in research work along the lines of the silkworm disease, and anthrax in cattle for the French government, succeeded in proving that the etiology of these diseases was due to micro organisms. Later he found and reported the cause of chicken cholera, and by inoculation experiments told how to guard against them. Since the fundamental work of Pasteur and Robert Koch, the studies of pathogenic micro organisms in general and of pathogenic bacteria in particular have been placed on a firm basis, and have assumed the greatest importance in the theory and practice of medicine. The connection of bacteria with certain forms of disease was conclusively demonstrated by these men, although it had long been suspected that suppuration was due to the presence of organisms in wounds.

That numerous small organisms scourged and ravaged the human body and that all people did not die during the epidemics of disease proved that the body protected itself against disease; this fact was laid bare to the knowledge of the world by the famous Erlich in his theory of phagocytosis, and side chain theory. He proved that the extensive metabolism of the human body was endowed with the power of eating or feeding upon the micro organisms which invaded the body. This substance which accomplished this was called phagocytes, taken from the Greek, *phagoto,* eat, *cyte,* a cell, are classed to day as leukocytes or white blood corpuscles. They have attractive powers and can remove from the blood the bacteria and foreign bodies of invasions, in the same manner as iron filings are attracted toward a magnet; the iron filings being the disease producing bodies and the magnet, the leukocyte. This is shown by the attraction or repulsion that exists between

leukocytes and foreign material in the blood stream, and when this attraction takes place as was demonstrated with the iron filings, it is called a positive chemotaxis. Numerous other observers realized that when an invasion of bacteria into a body had taken place successfully that some of the phagocytic cells had lost their power of eating, and destroying bacterias. This phagocytic power of the body being lessened, the power of the bacteria was increased, and the body was then in a diseased condition. In the accounts of Robert Koch we read that he took pure cultures of living micro organisms, and after introducing them into normal salt solution, killed them with heat, and injected them into a body that was not resistant to the disease produced by the micro organisms used. It was then claimed that after repeated injections, these animals would be inoculated with the disease, due to the fact that phagocytes had been injected by the invasion of dead bacteria.

This led up to the wonderful theory of Metchnikoff, which has continued to the present day. In 1883 Metchnikoff first claimed that phagocytes protected the higher animals against infection by disease producing bacteria, although for years he knew of the presence of phagocytes in lower animals, in the class of "Tunicata" called "Actinia." His experiments on higher animals were quite easy to be carried out.

By defibrinating the blood of a goose, by whipping it with a bundle of pieces of wood or wire he obtained a mixture of blood corpuscles and serum, (the fibrin was removed by the whipping) this defibrinated blood was mixed with physiological salt solution, centrifuged and the clear supernatant fluid pipetted off. In this way he obtained what is now known as "washed red blood corpuscles." A few cubic centimeters of these washed red blood corpuscles were suspended in physiological salt solution and were injected into the peritoneal cavity of a guinea pig, and after a short time removed from the peritoneum together with the exudate from the abdominal cavity with which they had been mixed. He found that the exudate contained numerous large mononuclear white blood corpuscles of the guinea pig, and that these contained many red blood corpuscles of the goose. Then at short intervals of time he removed more exudate for examination and found the goose

corpuscles more and more digested in the large mononuclear cells of the guinea pig, demonstrating that certain cells of the Mammalian animals possess phagocytic properties.

Metchnikoff's opponents tried to disprove this by showing that in animals dead from anthrax, numerous anthrax bacilli were seen in the blood, none of which were being or had been taken up or digested by phagocytic cells. Metchnikoff then succeeded in demonstrating that in animals not susceptible to anthrax such phagocytosis of bacilli took place, and that the lack of susceptibility depended upon the fact the anthrax bacilli were taken up and destroyed by the phagocytes. Upon this fact the use of vaccines in medicine is based, the vaccine, being the substance used to increase the phagocytic powers of the body.

Non-susceptibility to a given disease or a given organism or toxin, either under natural conditions or under conditions experimentally produced, is called immunity. Immunity is, in fact, of widely varying degrees and has correspondingly relative significance. So long as an organism continues to exist, it must continue to adapt itself to its invironments, and thus it becomes so modified as to effectually resist influences which, without such modification, would have brought cessation of being. The lower animals are immune to some diseases prevalent in man and certain families have marked resistance to some diseases. These are examples of natural immunity. An individual may be immune by virtue of his being of a certain race or family. Certain animals may possess a congenital natural immunity. For instance, many warm blooded animals, such as guinea pigs, cattle, mice, and rabbits, are susceptible to anthrax, while dogs and rats possess quite a strong, though not an absolute natural immunity against this infection. Man is susceptible to typhoid bacillus and cholera spirillum infections, while all our domestic animals are immune against these so far as natural infection is concerned. Classic is the observation, on the other hand, that one attack of a certain infectious disease affords lifelong immunity against attack of the same disease, while in other diseases the acquired immunity is varying in duration. Persons who have had one attack of measles, scarlatina, typhoid fever, and smallpox are generally

immune against a second. The same is true of animals having had hoof and mouth diseases. This is called natural acquired immunity.

We can produce artificial immunity either active or passive. Vaccination or the injection of bacterial toxins, produces active immunity, while the injection of an immunizing serum such as diphtheria antitoxin confers passive immunity. In other words in the first instance the patient supplies his own antibodies—active immunity (in the second instance the anti-bodies are supplied to the patient), passive immunity.

In active immunity, following recovery from either an idiopathic infection or an artificially produced infection, there are developed in the blood the anti-bodies which are inimical to the toxin or the activity of the bacteria of themselves, or which accomplish the destruction of the causative agent by the action of the phagocytes. Normal blood serum has a powerful destructive effect upon many varieties of bacteria, and this power is found to be greatly increased in a patient who has been infected with these bacteria, either naturally or artificially. There can be no doubt that in all cases of acquired immunity, either active or passive, the leucocytes have performed the large and important work of destroying and absorbing the process of phagocytosis.

Pathogenic bacteria secret very powerful soluble toxins which enter the general circulation; whenever such toxins circulate in the blood, there is a tendency to the formation of bodies which neutralize them, and bring about a cure of the conditions, provided that the toxins are not over abundant and have not already done irreparable damage. These bodies which neutralize the soluble toxins are called anti-toxins. The action of the anti-toxin upon a toxin, is best understood by comparison with the well known chemical reaction between acids and alkalies. Just as hydrochloric acid can be neutralized in a test tube with ammonia, so can a soluble toxin be neutralized with its anti-toxins. The principle is the same, although the process is much more complicated in the neutralization of toxin by anti-toxin than of acids by alkalis. The anti-toxin mixture can be injected into a susceptible animal without producing any ill effects. Thus the formation of anti-toxin is another means by which the body protects itself against pathogenic bacteria, and their most important products, toxins.

Wright and Douglas have demonstrated the presence of substances in the blood which act upon bacteria rendering them subject to phagocytes. The best known cause of phagocytosis, and the one occupying the attention of medical men almost exclusively, is the opsonin of the blood serum which was first clearly demonstrated by Wright and Douglas about 1907. Of several protective bodies known to exist in man in normal and immune seria, only the opsonins can be quantitatively determined with any considerable degree of accuracy. It should be remembered, however, that all the immune protective bodies arise from the action of the bacteria, and their chemical products; so that while the opsonins are distinct from others, the probable quantity of the others may be at least inferred from the amount of opsonin found present. Among the anti-bodies found in the serum, we have precipitins which are substances formed by the injection of protein solution and cause sedimentation or precipitation when the serum is mixed with a solution of the same protein as was injected. Agglutinins, which are formed as the result of invasion, or injection of bacteria; and the serum of an individual or animal so treated when brought in contact with the same species of bacteria causes them to collect into clumps; and lysins, the production of which is due within the serum of the recepters, which have the power of combining with the antigen, and also with the complement that exists in the serum of all animals, whether they are infected or not. In determining the amount of opsonin in a given serum, it is necessary to have (1) blood serum from a sick and from a healthy person, (2) leukocytes, (3) a suspension of the organism, the opsonin for which is to be measured.

The leukocytes are prepared by receiving a few drops of the blood in a normal salt solution with one per cent sodium citrate added. The mixture must be shaken and centrifuged at a moderate speed for five minutes. The leukocytes will be found in a grayish layer at the top of the sediment and may be pipetted off.

The blood serum from the person to be tested is obtained by bleeding into glass capsules or tubes and centrifuging.

Bacterial emulsion made from young culture of the required organism is made with 0.85 per cent salt solution: equal

parts of the leukocytes, blood serum, and bacterial emulsion are drawn up into a small capillary tube, and mixed in a watch crystal and again drawn into the tube. The tube is then sealed and incubated at blood temperature for about twenty minutes. The mixture is again shaken and smears made and stained with a good blood stain. Many of the bacteria will be found to have been assimilated by the leukocytes. The contents of a fair number, about a hundred, are counted and an average made. Simultaneously with this test, a control is made of a normal individual, one hundred leukocytes are counted and an average taken; the results of the latter divided into the result of the person being tested, give the opsonic index—i.e., if the normal one's average is four bacteria per leukocyte and the tested one is three per leukocyte, we would have three divided by four equals .75; in other words, three-quarters of the quantity of opsonins only are present in the serum of the tested patient.

The opsonic index is increased by the injection of toxins or bacterial bodies into healthy tissues, thereby the phagocytic power of the body is increased in proportion to the increase in opsonic powers.

Vaccines or bacterial vaccines or bacterins as they are now called, serums of anti-toxin, belong to the class of biologic preparations. These do not replace drugs, but are new means in treating diseases caused by bacterial infection, and are given with but one purpose in view, that is to produce immunity whereby the patient may be able to overcome and cease temporarily at least to be susceptible to attacks by pathogenic bacteria.

Bacterines consist of suspensions of killed bacteria. In the preparations of these no animals are needed. The bacteria for a specific bacterine or vaccine are grown on suitable media, removed from this media by washing with normal salt solution, killed by heat and then emulsified in salt solution.

The bacteria are then counted in order that a specified number may be administered at each dose and put up in suitable containers. Bacterines are used to produce active immunity when injected, they stimulate the patient's body cells to produce its own anti-bodies, including such substances as agglu-

tinins, lysins and opsonins. There is no doubt that the efficiency of bacterins depends upon their power to stimulate the formation of opsonins.

The serum or its modified and purified preparation, the globulin solution, is the older and best known of these two classes, i.e., bacterines and serums. The first successful serum, the diphtheria anti-toxin, was discovered by Behring and Kitasato in 1890. Anti-toxins are produced within the body of some animal, the horse being used in most cases. Such an animal is given injection of the toxins, rarely of dead bodies, of a specific disease producing micro-organisms in increasing quantities, until the point of tolerance or maximum resistance has been reached. As a result, anti-toxic substances are produced by these animal cells and they appear in the serum. At the proper time the animal is bled and after various stages of purification and concentration, the blood serum is put up in suitable containers for administration to the human patient.

This serum contains anti-bodies or immune substances which will directly combat the specific infection. By the injection of a serum the physician produces passive immunity. In order that a serum be of greatest therapeutic value it must be of a standard specified strength so that the size of the dose administered may be regulated. This process of regulating serums and bacterines is termed standardization, and in the case of the anti-toxin serum it is done in terms of "anti-toxin units." To test the anti-toxic value guinea pigs of about 250 grams are used. These guinea pigs and the parents of these guinea pigs should never before have been used in the testing of anti-toxin. An anti-toxic unit is to be understood by its effect only.

A unit is capable of neutralizing an amount of toxin, or bacterial poison; that is, in turn, measurable by its fatal effect on guinea pigs in the presence of a standard immunity unit furnished by the United States Government. The immunity unit is mixed with the toxin and administered to guinea pigs. Sufficient toxin must be used to kill the guinea pig notwithstanding the protection afforded by the immunity unit. One anti-toxic unit will just save the life of the guinea pig when injected together with the toxin dose above mentioned.

A short time after an inoculation is made, the opsonic index falls lower than it was previous to the injection of the vaccine. This was named by Wright the negative phase. Shortly after, from a few hours to several days, the opsonic index will rise above the starting point. This is called a positive phase. The amount of opsonins in the blood remains stationary for a variable length of time, and then diminishes. As soon as this diminution is noticed, a second injection of the vaccine should be administered. This second negative phase produced will be less marked than the first, and soon the positive phase comes on, reaching a higher level than that previously. Thus, the injections are repeated from time to time, according to the opsonic index of the patient's blood, and the positive phase attains a higher and higher level until it may be as high or considerably higher than that of a normal person. In other words, if vaccinations are properly given (never during a negative phase,) and as a result the patient's tissues are stimulated in the increased production of opsonins, phagocytosis is increased, and the patient rapidly recovers from his infection because the invading bacteria are disposed of. This is the principle mainly to be remembered for successful application of bacterines or vaccines in infectious diseases caused by pathogenic bacteria.

There are today a great many bacterines manufactured for medical use. Chief among them are the anti-rabic vaccine, the typhoid, pertussis vaccine, meningococcus vaccine, gonococcus vaccine, acne vaccines, anti-streptococcic vaccine, pneumococcus vaccine, staphylococcic vaccine, and a class of important vaccines which come under the head of tuberculins.

Wright carried out very extensive experiments in South America with the pneumonia vaccine. He worked under great difficulties and had great trouble in regulating the sizes and frequency of doses. His main guide in the choice of doses and intervals of administration was the ups and downs of the temperature chart, and clinical symptoms. Of one hundred fifty nine cases given the vaccine treatment fifty died, and of one hundred and forty given the expectant treatment, forty-eight died. In his succeeding experiments, larger doses of vaccine were administered and the percentage of death decreased.

Prophylactic immunization in scarlet fever is still an unsettled point. For some time streptococcus vaccines prepared from cultures isolated from scarlet fever patients have been used in Russia with a moderate amount of success. Later reports show that the results are about uniformly favorable, and the use of this vaccine is free from danger during the last couple of years. Dr. Schultze of New York has obtained very interesting results by using vaccine of a large diplococcus found in connection with scarlet fever.

The use of vaccines in the treatment of chronic diphtheria carriers has been a subject of much research work with a fair amount of success. This work was prompted by the great inconvenience caused to people called diphtheria carriers, who although they showed no signs of illness, were isolated because of the presence of diphtherial bacteria in their throats.

The treatment of pneumonia by vaccines has been successful. In this instance polyvalent vaccines were employed. In 1912, active immunity as well as passive immunity was obtained against the pneumococcus with a soluble vaccine.

Sensitized vaccines have again been brought into the limelight. After many unsuccessful applications of this class of vaccines they were for a time abandoned. In 1914 a sensitized vaccine virus was successfully made, and a case of uterine abscess was successfully treated by this sensitized vaccine of proteus.

An interesting application of vaccine therapy has come up for consideration in the last few years in the Hodgkins disease (pseudoleukemia). The status of the prophylactic vaccination against whooping cough is still an unsettled matter. This is due largely to the fact that small amounts of the vaccine are administered. There is little doubt that the use of vaccines in doses of one hundred million or more will remove all doubt as to its efficiency in controlling epidemics.

The tuberculins, which are divided into a boullion filtrate, concentrated, a dilute, a bazillen emulsion, and ointment for Moro test, tuberculins for von Pirquet test and purified tuberculin discs for ophthalmic tests and tuberculin old are supplied in both the human and bovine types.

Tuberculin old is used chiefly as a diagnostic agent. Tuberculin concentrated is a toxin and produces immunity to the toxin of the bacillus, and not to the bacillus itself. Tuberculin boullion filtrate is used in the treatment of tuberculosis and is administered in doses ranging from 1/100,000 to 1/1000 milligram, the dose is repeated every three to six days.

The use of vaccines is becoming greater and their application wider as time goes on. It is the ultimate hope of physicians to use bacterial products in the fight against Pathogenic Bacteria. One great stumbling block has been the tendency to use small doses; this has been overcome to a great extent. The addition of a vaccine to an infected person by injection throws into the body a great number of poisons. The physician must therefore be very careful in the administration of the initial dose. He must also know whether to administer a serum or a bacterine. A bacterine should be given in cases of localized infection, at the beginning of an acute disease, in chronic infectious diseases and for prophylaxis against typhoid fever and cholera. Serum should be given for immediate prophylaxis against diphtheria, in general infections fully developed, and when on account of the severity of the symptoms, an immediate response is essential.