After many experiments the following method was found to yield the most satisfactory results. Cylindrical jars of one litre capacity are used, and vaseline is thoroughly applied to the interior to prevent adhesion of the clot to the sides. A large test-tube filled with cracked ice, closed at the top by a perforated rubber stopper, is placed in each jar before receiving the blood. When filled the jar is placed on ice, and the blood slowly coagulates, becoming a firm mass in the course of several hours. The test-tube is then withdrawn from each jar, leaving a cylindrical deficiency in the clot. At the end of twenty-four hours the result is striking. The clot has shrunk to a soft, flabby mass, and is surrounded by abundance of clear yellow serum. This is removed by carefully pouring off, and constitutes about two-thirds of the total amount obtainable. If the jars are placed on the ice another twenty-four hours, about one-third more may be obtained. This remainder is colored by solution of pigment in varying degrees, but is equal in all therapeutic qualities to the first yellow serum. The strength is ascertained by either the French or German method. The former was adopted at the beginning of the work, but I have recently come to regard the latter as more scientific and accurate.

The serum is next passed through a Pasteur filter to ensure sterility, and the filtrate shaken with the chloroform-thymol solution previously mentioned. It is, however, not allowed to stand long in contact with an excess of the solution, since a precipitate readily forms, but is immediately transferred to sterilized bottles, and aseptically sealed, being then ready for use.

DIPHTHERIA AND THE ANTITOXIN TREATMENT.¹

BY W. F. ROBIE, A.B., M.D., BALDWINVILLE, MASS.

THE current medical literature is super-saturated with antitoxin. Many times are given the mode of manufacture, the experiments on the lower animals, the way of using for diphtheria in children, statistics showing the results of its use and its probable great value as a remedial and preventive agent. Little is said in connection with all this of the comparatively new science of bacteriology of which antitoxin is the last and greatest discovery and the greatest achievement. From lack of experience I am not at all fitted to discuss the advantage of the new over the old treatment for diphtheria. I am but little better prepared to give briefly the facts in the science in the order of their discovery, which have made antitoxin possible. This latter, however, I have attempted to do, feeling the need of renewing my acquaintance with some of the elementary facts in bacteriology and feeling the need of gaining at least an understanding of the most important parts of the recent work in this line, hoping all the time that some of you, perhaps all, feeling this need to some degree, may be interested in following me. I shall give, at the out-start, some definitions which seem to me to be so axiomatic as to need no discussion or qualification. If you are disposed to argue them, their brief statement may seem dogmatic; if you already know them, it may seem unnecessary that they are stated at all; but this seems to me to be

the only way to present the whole subject briefly enough for this occasion.

Bacteriology is the science of bacteria. Bacteria, or germs, are microscopic members of the vegetable kingdom. There are several principal varieties, classed according to their form: micrococci, or round bacteria; bacilli, or rod-shaped; spirilla, or spiral-shaped. Streptococci, or round bacteria connected like the links of a chain; diplococci, or round bacteria in pairs; and staphylococci, or round bacteria clustered like grapes, are various arrangements of micrococci. Some bacteria live in the air, are aërobic; some live in the absence of air, are anaërobic; some thrive on dead matter, are saprophites; some thrive on living matter, are parasites. An obligate saprophite is obliged to live on dead matter; "their number is unlimited and they are the scavengers of creation." A facultative saprophite has the faculty of living on dead matter under favorable circumstances, though by nature parasitic, for example, the tubercle bacillus of Koch. An obligate parasite is obliged to live on living matter, for example, the germs of syphilis and leprosy; and a facultative parasite has the faculty of living on living matter, for example, the anthrax bacillus.

Bacteria multiply in two ways: (1) By fission, which is simply division, one bacterium forming two or more. Bacteria multiplying in this way are quite readily destroyed. (2) By spores, in which the spore or bud develops at some part of the parent bacterium, and when sufficient development is reached becomes detached from the parent. Bacteria multiplying in this way are destroyed with difficulty.

Pathogenic bacteria cause disease. Non-pathogenic bacteria do not cause disease. Many diseases are caused by bacteria. To prove that a given bacterium produces a given disease we must conform to the four rules of Koch, and show (1) that the bacterium is present in every case of the disease; (2) we must isolate the bacterium; (3) we must produce the disease in healthy animals by inoculating them with this bacterium; (4) we must then find the bacteria growing and working the special changes in these animals.

Diseases already proven to be caused by bacteria are tuberculosis, tetanus, anthrax, glanders, progressive edematous gangrene, gonorrhea, croupous pneumonia, diphtheria, etc. Diseases practically proven to be caused by bacteria are typhoid fever, Asiatic cholera, malaria, influenza, etc. Diseases which we have many reasons to believe are caused by bacteria are syphilis, leprosy, meningitis, erysipelas, septicemia, puerperal fever, measles, scarlet fever, whooping-cough, small-pox, yellow fever, etc.

Different bacteria produce different products, some poisonous, some non-poisonous. The tubercle bacillus produces tuberculosis. The Klebs-Löffler bacillus produces diphtheria. The yeast plant or germ produces alcohol. It was early observed, that, in this latter case, when the yeast plant had formed a twelve-per-cent. solution of alcohol, the plant died and made room for the germ which produces acetic acid. In time this germ died and another took its place, and so on, each germ being destroyed by a poison formed by itself.

From these and other observations a truth has been discovered, namely, that every living thing, plant or animal, may be poisoned by the products of its own metabolism. This fact has been the basis of the

¹ A paper read before the Miller's River Medical Society.

bacteriological treatment of disease. After many theories and much contention it has been settled that bacteria produce disease by forming, in the process of their life changes, poisonous chemical compounds. Some years ago certain of these were isolated and they were named ptomaines or putrefactive alkaloids. After a time it was discovered that other poisons were formed, more obscure in nature but much more virulent than the ptomaines. In recent years much time has been spent in attempts to discover these unknown poisons, and Hankin, an Englishman, succeeded in isolating the first poisonous albumin of known bacterial origin from cultures of the anthrax bacillus, though poisonous albumins had been discovered some time previously by Mitchel and Reichert. Many of these albuminoid bodies have now been isolated; and, though little is known of them, we know that they are all intensely poisonous and that they will, when injected into an animal, cause some or all of the symptoms of the disease of which the germ from which each albumin is derived is the cause. Such is the toxin of diphtheria.

It has long been known that some animals are not susceptible to certain diseases, and that some diseases, particularly the acute infectious diseases, leave the one that has suffered from them proof for a longer or shorter time against them. It had been known since antiquity that one attack of small-pox gave immunity against subsequent attacks; and when, in 1795, Jenner discovered vaccination, this gave rise to much thought and experimentation for the purpose of finding out in what way vaccination gave protection against small-pox. To make the question general, Wherein is the power which protects one animal from a disease which other animals are subject to, and what is the reason that one attack of certain diseases confers immunity against subsequent attacks? At first a partial explanation was based on the fact that certain cells — leucocytes or phagocytes — were more numerous in one animal than in another. These cells were thought to be so numerous in some cases that they could absorb all the poison or destroy all the bacteria which gained admittance to the body. Later, the blood of some animals not susceptible to anthrax was freed of all cells of every kind, and the fluid was found to kill the anthrax germ. In the case of animals susceptible to the disease the germs would thrive on the fluid elements of their blood. This proved that the germicidal properties are present chiefly in the fluid elements of the body. When this fluid was heated to 60° or 70° F. all germicidal properties disappeared at once. The fluid being largely albuminous, it was conjectured that the heat had coagulated the albumin, and that this albumin in its natural state had been the protective element.

It is now proven beyond a doubt that the albumins or albuminoid substances possess this protective power, or, in other words, the susceptibility of an animal to a germ disease depends upon the relative amount of certain albumins in the blood. Acting on the generally known fact that one attack of an infectious disease gives immunity for a longer or shorter time against the disease, the foremost investigators sought to give protection by producing in the person a mild form of the disease, just as, for centuries, people had sought to avoid a severe attack of small-pox by inoculating themselves with the poison during a mild epidemic.

Pasteur secured immunity in fowls against chicken-cholera. He allowed the bacteria to grow on gelatine in a tube to the thirtieth generation. He then injected into the tissues of the fowl this modified form of the bacterium, and produced a form of the disease so mild as to be entirely without danger, but sufficient to protect against future attacks of the disease. He tried to produce immunity against anthrax in the same way, but failed. Later, by allowing the bacterium to grow at a temperature above 42° C. to the twenty-fourth generation, he obtained a substance which he called primary vaccine, which, when injected beneath the skin of the animal, produced a slight effect. He followed injections of this in a few days with injections of a secondary vaccine, or what he obtained after allowing the bacterium to grow to the twelfth generation, and this gave protection against the inoculation form of the disease. His next experiments were for the purpose of finding a protection against hydrophobia. Knowing that the lesions of the disease were in the brain and spinal cord, he made an extract of the fresh cord of an animal dead from hydrophobia, and injected this extract beneath the skin of another animal. This produced the most virulent form of the disease. He finally secured a protective vaccine in this way. He dried for forty days, in the air, the spinal cord of an animal dead from this disease. The cord of another dead from the same disease he dried thirty-five days; of another, thirty days; and so on. He then made separate extracts of the dry cords. He then injected beneath the skin of the animal which he wished to protect some of the extract of the cord that had been dried forty days; in a few days he injected some of the extract of the cord dried thirty-five days; and so on, till at last he injected an extract of fresh cord. The animal was thus accustomed to the poison and, after the last injection, was proof against the disease. This is the principle of the successful preventive treatment of hydrophobia used at the Pasteur institutes in Europe and in this country.

Brieger isolated from cultures of the tetanus bacillus a toxine which, when injected, produced the symptoms of the disease. An antitoxin has also been discovered in the blood of animals which have had the disease. If such blood be injected into animals suffering from tetanus, if it be early enough in the course of the disease, they will get well. It will also confer immunity against the disease in the animal receiving it.

Many other experiments have been conducted on these lines, with varying results; but by far the most sensational and the most widely known were those of Robert Koch, with the substance known as tuberculin. Precisely the same principle is involved here, and the tuberculin is made by growing the tubercle bacillus under unfavorable conditions, in the presence of moisture or under pressure or with varying degrees of heat and cold. The culture is filtered, and the filtrate is the tuberculin. In treating some diseases or in conferring immunity against them, it has been the custom to inject a modified culture of the bacillus, thus allowing the bacillus to grow and to develop in the organism, making the changes in the blood which would render the person immune; and in some diseases, not the bacteria themselves but a modified and weakened form of the poison obtained by filtering a culture of the organism is repeatedly injected until the system becomes saturated with the poison. Then if the bacteria

gain admittance they cannot live, or, if they are already present, they are destroyed by the antagonizing power of the poison. Koch followed the latter method, and the fact that the remedy has failed to meet his expectations and those of the medical profession, and that it did, for a time, apparently place discredit upon the whole science of bacteriology, was not due to wrong methods but to the nature of the disease with which he had to deal. Tuberculosis is characterized by the formation of a degenerate form of tissue known as tubercle. Tubercles are full of the characteristic bacilli constantly producing their poisons. Tubercles are continually breaking down and the dead tissue, mingled with the poisons formed by the bacilli, goes into the circulation, producing the constitutional symptoms — exhaustion, emaciation, hectic fever, etc. When there is injected a poison like tuberculin, though it may be directly opposed to the poisons in the system and able to destroy the pre-existent poisons under proper circumstances, will it not, since it must cause a reaction and a sloughing of more tissue and cause the blood to be more completely saturated with the very elements, dead tissue and the germ-produced poisons, which had brought on the constitutional symptoms of the disease in the first place — will it not, in reason, often cause an increase in these symptoms and, at times, collapse? At any rate such has been the case, and, though tuberculin has been classed as a failure as a cure for tuberculosis, it has shown to scientific workers the limitations of the old bacteriological methods of treatment and has awakened the most enthusiastic efforts to find a safer and more sure treatment involving the same fundamental principles but going one step further. It was found to be a dangerous process to antagonize in the human system the growth of poisonous bacteria by the introduction of poisons deadly to the bacteria.

I will re-state that the germicidal elements in an animal are chiefly in the blood. An animal can be made proof, for a time at least, against a bacterial disease by having the disease and recovering; for the bacteria produce and leave in the blood the poisons which are deadly to themselves; or we may accomplish the same thing by giving the animal subcutaneous injections of the poison made by the bacteria in the course of their growth upon various culture media. Both of these methods are, as we have seen, at times dangerous. Fränkel first secured immunity against diphtheria by injecting beneath the skin of animals a substance prepared from cultures of the bacillus.

Behring, the discoverer of antitoxin, began his experiments some years ago. While I was in the medical school I learned that he had isolated two albuminoid substances from cultures of the Klebs-Löffler bacillus; and that he had found that ordinary cultures of the diphtheria bacillus heated to 60° or 70° F. and then injected beneath the skin of animals had the power of conferring immunity; and that he had also found that the addition of iodine trichloride to old cultures of the bacillus also gave immunity when injected subcutaneously. If he had stopped here, we should have had for diphtheria a remedy analogous to tuberculin for tuberculosis; but he went one step further. He injected subcutaneously the poison or toxine made from cultures of diphtheria bacilli into various animals. Large quantities injected at first would kill the animal, but small quantities repeated

from time to time would be borne, and after a time the animal became accustomed to the poison and larger doses could be used. This was kept up for months. Then blood was taken, and the serum was injected into animals suffering in varying degrees from the diphtheritic poison, or into animals exposed to the poison. He found that animals which had received injections of this blood serum in sufficient quantities would not develop diphtheria under any circumstances for some time after the injections. This immunity would always be temporary, but would last from several days to several weeks, according to the amount of serum injected. The amount of serum which would protect a guinea-pig would not protect a rabbit. This showed that the serum did not destroy the toxine, but either rendered the cells of the animal insensible to its action or made their power of resistance to it greater. When the poison was injected alone, there were diphtheritic symptoms and death. When a mixture of serum and poison in right proportions was injected, there was no effect, or but a slight fever. When the poison was previously injected and the disease well developed, recovery generally resulted if the serum was injected repeatedly.

To approach as nearly as possible to diphtheria in the child, Roux and others inoculated the mucous surfaces of various animals with the germs of diphtheria. Practically the same results were obtained. When serum was injected before inoculating with the germ there would be local redness, fever and some general disturbance; but no membrane would be formed. When the serum was injected after the formation of false membrane, the swelling began to diminish in a few hours; the fever abated on the following day in moderate cases — after repeated injections in severe cases — and the false membranes were detached during the first or second day. When the trachea of a rabbit was inoculated with the bacilli of diphtheria, there was, within forty-eight hours, fever, false membrane formed and difficult breathing. If the serum was injected before the germs, there were none of these symptoms; or if it were injected in too small quantities, there were late symptoms. If the serum was injected after the membrane was developed, the disease was arrested. When streptococci and other toxic bacteria were present with the diphtheria germ, the disease as had been previously noticed by others, was found to be more severe, as the diphtheritic poison was increased, and there was a tendency to broncho-pneumonia. If the serum was injected early after the mixture of diphtheria bacilli and streptococci, a portion of the animals were saved; but if there was much delay the disease terminated fatally.

In preparing the serum for man the horse was found to be the best animal to accustom to the poison or toxine. Young and healthy animals are selected; and they are so little susceptible to the toxine that there is little danger of killing them if moderate care is taken. The toxine is prepared by growing the Klebs-Löffler bacilli in a medium of peptonized broth. The cultures are made in flat-bottomed glass flasks into which a current of moist air is passed. The temperature must be 37° C., and the time required is at least three weeks. The cultures are then filtered, and the filtrate, to which is added a little camphor, is the toxine ready for use. From two to five cubic centimetres of this are used as an initial dose. A larger dose proportionately can be used if the trichloride of iodine is mixed with the

toxine. This is injected subcutaneously. The horse is given another dose in a few days and the amount is gradually increased until he can bear large quantities of the poison. Four months or more is necessary before a very active serum can be obtained. Then blood is drawn from the jugular vein, and the serum separated from the other elements. When a little trikresol is added to it, we have the antitoxin ready for use.

The serum obtained from the horse is now tested as to its strength. It is first found how much toxine is necessary to kill an animal of a known weight. Then it is found how much antitoxin is needed to bring about a good recovery, or to neutralize the effects of the toxine in an animal of the same weight. The amount of the antitoxin taken, divided by the weight of the animal, gives the strength of the antitoxin; for example, if one cubic centimetre is used and the animal weighs 1,000 grammes, the strength of the solution is 1 to 1,000, or one part of serum to one thousand of body weight is needed for a curative dose.

In treating diphtheria in man the serum is injected into the tissues of the flank or between the shoulders. A large hypodermic syringe is used, preferably one made with fittings of asbestos and linoleum, and holding from 10 to 20 c. c. Before using, this should be sterilized by boiling. The serum should all be injected in one place, and there should never be massage of the part after the injection. Different operators use a different initial dose. As the serum varies so much in strength, it is hard to tell the dose unless the strength of the serum is known. For a child moderately sick, 10 c. c. of the stronger serum seems to be the most common dose; for one seriously sick, 20 c. c. If no improvement is noticed the dose may be repeated in twelve hours, and in some cases the amount is increased. One child received 125 c. c. of the serum with no bad effect.

Diphtheria, as you all know, is an acute infectious disease caused by the action of the Klebs-Löffler bacillus. It is primarily a local disease, cell proliferation and fibrous exudation brought about by the action of the bacillus at the point of invasion going to such an extent that death often results from the mechanical closing of the air-passages. It is secondarily a constitutional disease, the poison formed by the bacillus entering the system and producing many serious consequences — albuminuria, heart-failure, paralysis, etc., and often death. The treatment, formerly expectant and symptomatic, has, in recent years, been rigidly antiseptic and symptomatic. Under the latter treatment are included antiseptic washes and sprays locally, such as hydrogen peroxide, bichloride of mercury, boracic acid, etc., intubation or tracheotomy when necessary, perfect rest, liquid diet, empirical agents, such as mercury in some form and tincture of the chloride of iron, and tonics and stimulants in every form. Under the old treatment the mortality was terrible, varying from 50 to 90 per cent.; under the later treatment, though somewhat reduced, it was very high, varying, in general terms, from 30 to 70 per cent.

So far, under the treatment by antitoxin, we have the almost universal testimony to the following remarkable results. If the disease has not advanced too far, the false membrane ceases to spread within twenty-four hours after the time when the antitoxin is injected, and the membrane becomes detached within forty-eight

hours, as a rule. Therefore, tracheotomy is less often necessary, intubation for twenty-four hours usually being all that the worst cases require. In mild cases the temperature falls very rapidly the day after the injection. In severe cases it begins to fall after the second or third injection and continues to fall slowly. The pulse-rate is lessened, but the action on this is slower than on the temperature. The albuminuria is much less frequent than with any other treatment, and when present is much milder in character. The other complications and sequelæ are more rare and less apt to prove fatal than with any previous treatment.

I have not time to discuss at length the statistics so far obtained. That we must put some confidence in them is certain from the character and scientific reputation of the observers. Some have used the serum in severest cases only. Some have used it in all cases. But all the statistics given, or nearly all, are for cases of true diphtheria, as proven by the presence of the Klebs-Löffler bacillus, and many had complicated with this germ the staphylococcus pyogenes or a streptococcus. These complications are known to be more severe than when the diphtheria germ alone is present. Statistics have appeared in the best medical journals above the signatures of reliable observers, and we have every reason to believe that each observer has made an accurate record of his work. Still the time has been too short to allow of our fixing definitely the future status of antitoxin at this time.

With this history of the progressive work in this wonderful new science of bacteriology before us, and with these eloquent figures, shall we not believe that there has been discovered the germ, at last, of all future treatment for diphtheria and all allied diseases? Reasoning from analogy, shall we not believe that, in the near future, the tuberculin of Koch, or some similar substance, will be used to immunize animals, and that their blood serum will be used in the prophylaxis and in the treatment of the early stages of tuberculosis in man? I believe the time is not far distant when, so far as the germ-produced diseases are concerned — and these diseases will then have been proven to be by far the most numerous and the most dangerous — we shall have, as our homeopathic friends now have, explicit directions in regard to the use of a certain remedy in certain proportions for a certain disease — not in such infinitesimal quantities that the wildest imagination can hardly conceive of the remedy as being present at all, but in definite quantities of a known strength, which will give a definite, satisfactory result when the disease is recognized and the remedy applied in time.

Medical Progress.

REPORT ON CHEMISTRY.

BY WILLIAM B. HILLS, M.D.

ALBUMINURIA.

GLOBULIN is usually associated with albumin in the urine. According to Boyd¹ the proportion varies so much that it is not possible to determine the variety of kidney disease by means of it. Even in amyloid degeneration, the globulin may not be in excess. In the albuminuria of pregnancy, the globulin is present in

¹ Journal of the Chemical Society, London, March, 1895, ii, 82, from Rep. of Lab. R. C. P., Edin., 1894.