say that we have found a practical way to grow belladonna from seed that eliminates the difficulties of field sowing. The various details regarding the germination of the seed have been worked out. Furthermore, the abundant material on hand made it possible to study thoroughly the distribution of the alkaloids in the various parts of the individual plant; the development of alkaloids in the seedlings and early stages of growth and the relative concentration of alkaloids in the leaves with relation to age and size.

As has already been said, belladonna was chosen for this work for certain reasons. If the problem is finally brought to a successful conclusion its value will lie not so much in what has actually been done with one plant as in the fact that it points the way to the possibility of a broader application of similar methods to our field of medicinal plants.

SENSITIZED VACCINES.*

F. E. STEWART, PH. G., M. D.

Sensitized Vaccines, Sero-Vaccines, or Sero-Bacterins, are suspensions of pathogenic bacteria, living or dead, artificially sensitized by treating them with immune homologous serums, i. e., serums from animals immunized against bacteria of the same kind as those used for producing the Vaccines. By this means the amboceptors contained in the immune serums are made to combine with the bacteria and sensitize them, so when they are injected into the body the complement and phagocytes normally present in the blood of the injected individual immediately combine with and digest them, and the resultant products stimulate the tissue cells to produce antibodies to which the subsequent immunity resulting from the vaccination by the sensitized vaccine is due.

How the Immune Serum is Obtained.—The immune serum for making sensitized vaccines is usually prepared by treating goats intravenously, first, with dead, and later with living cultures of bacteria. Trial bleedings are made at regular intervals, and the serum is tested for amboceptors and other specific antibodies. When the serum shows a sufficiently high titre, a large quantity of blood is withdrawn for use in preparing the sensitized vaccine.

How Vaccines are Sensitized.—The bacterial cultures to be sensitized are added to a little physiological salt solution, emulsified, turned into a vessel containing the immune serum, allowed to macerate for a few hours, the clear and slightly opalescent liquid separated from the deposit of bacteria, and the latter washed by centrifugalization several times in physiological salt solution until the last traces of serum disappear. The white mass thus obtained is of a pasty, semiliquid consistency, and after standardization by bacterial count, and the addition of physiological salt solution in proper amounts, produces an entirely homogenous emulsion which constitutes the sensitized vaccine.

*Read before the Seaboard Medical Association, Norfolk, Va., December 9, 1913.

How Sensitized Vaccines Were Introduced to Science.—Sensitized Vaccines were introduced to science in 1902 by Besredka, a scientist connected with the Pasteur Institute, and have gradually and progressively attracted as knowledge concerning them has been developed by Besredka and his associates. Among the latter are Garbat and Meyer, Gordon, Broughton-Alcock, Boinet, Cruveilhier, Bertrand and Feigan, and other investigators. The researches have been carried on at the Pasteur Institute and l'Hotel Dieu, Paris, also at St. Bartholomew's Hospital, London, and other well-known institutions. One of the sensitized vaccines, that is used for immunizing against bubonic plague, is prepared from killed sensitized plague bacilli, and is now official in the French Codex.

What is Meant by Sensitization.—Normal blood serum, owing to the alexin or complement it contains, and the small amount of natural amboceptor present in the blood, possesses in some degree the power of digesting bacteria and other protein substances. This power is enormously increased during the process of parenteral digestion. This is due to the stimulating effect upon the tissue cells of the protein introduced. For, if a protein is introduced into the tissues, the tissue cells are stimulated to produce a specific amboceptor, the function of which is to sensitize the protein and thus prepare it for digestion. This digestion is accomplished by the joint action of amboceptor and complement. (Vaughan says that all digestive enzymes are composed of amboceptor and complement.)

But the complement cannot act upon the protein until the latter is first sensitized by the amboceptor. Amboceptors are *specific,* and the kind of amboceptor produced by the tissue cells depends upon the kind of *antigen* (protein) used to produce it. The digestion is called *lysis.* Hence we have bacteriolysis produced by the joint action of specific bacteriolytic amboceptor and complement upon the kind of bacteria injected into the tissues.

When unsensitized killed bacteria (bacterin) are injected into the tissues, all of this complicated process of parenteral digestion must be carried on by the body cells to produce the specific amboceptor and other antibodies to which the value of the bacteria as an immunizer is due. In preparing sensitized vaccine, the bacteria are artificially sensitized by the amboceptor in the specific (homologous) serum before injection, and thus prepared for the immediate action of the complement already present in the blood of the individual injected.

For What Sensitized Vaccines are Used.—Sensitized vaccines are used against typhoid fever, rabies, plague, cholera, and other infectious diseases, and are also employed in their treatment. Several thousand people have been immunized by their use, and they have also been quite extensively employed as therapeutic agents by many competent observers. Further researches are necessary to determine their merits in comparison with bacterial vaccines prepared by the Wright method, also to ascertain the comparative value of sensitized vaccines prepared by sensitizing living cultures and cultures killed by heat.

How Sensitized Vaccines Act: Garbat and Meyer's Explanation.—In explaining the action of sensitized vaccines, Besredka refers to the researches of Garbat and Meyer. These investigators claim that bacteria are typical cells consisting of an external protoplasmic envelope and an internal nuclear portion. When they are disrupted by the action of amboceptor and complement, the outer portion is

digested and the inner portion set free. Both portions are toxic; both give rise to individual immunizing substances by stimulating the tissue cells to produce them.

Vaughan's Explanation.—As the explanation of Garbat and Meyer resembles in some particulars the teachings of Vaughan and his associates, I wrote to Professor Vaughan in regard to sensitized vaccines, and asked further information on the subject from his view-point. His reply proved very interesting to myself and friends, and became the subject of considerable debate. Thinking that you might be interested in Professor Vaughan's letter and also in the debate following its reading, I am now presenting both to you for consideration. My paper also contains valuable information taken from the papers of Besredka and his followers, and pertaining to the subject before us:

ANN ARBOR, MICH., November 8, 1913.

DEAR DR. STEWART—It seems to me that the action of sensitized bacteria compared with unsensitized bacteria is best explained by my theory. Probably it will be best to first state my theory and then see how it applies to sensitized bacteria. A protein sensitizer or anaphylactogen (called by others antigen) is a protein substance which when injected into animals causes certain body cells to produce a specific proteolytic ferment. This specific ferment digests and destroys its homologous sensitizer or the protein which has caused its development. This ferment, like all other ferments, consists of amboceptor and complement. Now let us apply this to sensitized bacteria. Bacteria, typhoid bacteria for instance, are sensitized by submitting them in vitro to immune serum. These bacteria thus are saturated with their specific amboceptors and when such sensitized bacteria are injected into an animal they are already fitted for complete digestion. In the animal the complement acts upon the prepared bacteria and their digestion is complete. So complete is their digestion that a large part of their poisonous constituents is destroyed and the animal is immunized by the non-poisonous constituent of the sensitized bacteria. For this reason the animal treated with sensitized typhoid bacteria shows little disturbance, while on the other hand, the animal treated with unsensitized bacteria must elaborate both amboceptor and complement. This takes time, the period is longer, the digestion is less complete, more of the poison is set free, less of the poison is destroyed in the process of digestion, and consequently the life of the animal is placed in geater jeopardy. Garbat and Meyer believe that immunization is secured only by the poisonous constituent, or constituents of the typhoid bacillus. According to my theory, the poisonous constituent of the typhoid bacillus has nothing to do with the production of immunity or sensitization. Sensitization and immunity are induced by the non-poisonous part of the typhoid bacillus. Subjecting the typhoid bacillus in vitro to immune sera, in other words, sensitizing the bacteria in vitro, prepares the bacteria for digestion, and when introduced into the body they are digested speedily and completely, or so nearly completely that a large part of the poisonous part of the bacterial molecule is destroyed. It seems to me that if the article by Garbat and Meyer is read with my theory in view, it is confirmatory of that theory. It has been shown by Friedberger, myself, and others, that very small amounts of the protein poison produce an elevation in temperature. Large amounts produce a depression in temperature. Sensitizing with immune serum in vitro prepares these bacteria for ready and complete digestion as soon as they are introduced into the animal body. Therefore, there is less disturbance in the animal body when sensitized bacteria are introduced than when unsensitized bacteria are given. This is the way I look at it.

I don't know whether I have made myself clear on this point or not. I know that I have been able to sensitize animals with the non-poisonous part of typhoid bacteria. This non-poisonous part which I have obtained has been secured by a crude method. The non-poisonous part which is split off by sensitizing bacteria with immune serum is a much more efficient preparation than mine. The point that I insist upon is that the sensitizing group in the protein molecule, and this of course means the immunizing group, is not the poisonous group, but is found among the non-poisonous groups. The poisonous group in all proteins is much the same, physiologically the same, chemically there must be fine differences, while the sensitizing group is not the same in any two kinds of proteins; hence its specificity.

I may be cranky on this subject. I think that the nomenclature of Ehrlich has been wrongly applied to sensitization and to bacterial immunity. The protein poison is not a toxin; it is a poison. It produces no antibody. Yours,

V. C. VAUGHAN.

Parenteral Digestion in Relation to Infection and Immunity and the Action of Vaccines.—This letter of Vaughan becomes far more interesting after reading

his most instructive book entitled "Protein Split Products in Relation to Immunity and Disease."[1]

In his book he explains how the tissue cells of the animal body have the power of digesting and producing enzymes for so doing. Digestion carried on outside of the alimentary canal by the tissue cells is called by Vaughan "Parenteral Digestion."

The questions arise why bacteria, many kinds of which live as commensals (at the same table) on the skin and mucous membranes of the body, feeding as saprophytes on dead matter, including worn-out epithelial cells, excrementitious matter, particles of food, etc., do not attack the living tissues and become parasites? And, in case they do become parasites, and obtain their sustenance by preying upon the tissues, how does the body get rid of them?

Vaughan attempts to answer these questions in his way, Metchnikoff has another way of answering them, Ehrlich another, Wright another, Besredka another, and so on. Each of these views resembles each other in many respects, and diverge in others. They cannot all be harmonized and I shall not attempt in this debate to harmonize them. But there are certain points which need connecting to make the subject before us intelligible to those who have been following Wright in his theories of infection and immunity, and employing Wright's bacterial vaccines in their practice. Besredka's sensitized vaccines appear to be a marked improvement upon Wright's products, and it is an important matter for us to decide whether they are or not. Let us therefore consider the theories underlying the subject, hoping to get a clear idea of Besredka's claims and the investigations upon which they are founded.

In answer to the questions, why do not the bacteria living with us as commensals become parasites, and when they do become parasites, how does the body get rid of them, all authorities agree in the following answers: The tissues of the body possess natural resistance to the action of enzymes, including the action of the enzymes secreted by the bacterial cell. This resistance, when especially marked, is called *immunity*. When bacteria succeed in overcoming this resistance, and invade the body as parasites, the tissue cells secrets specific enzymes which digest and destroy them.

According to Vaughan, infectious diseases are groups of symptoms caused by the parenteral digestion of bacteria, especially by the splitting up of the bacterial protein. This, Vaughan teaches, is split into several portions, viz., a primary chemical group, or archon (keystone), with which are connected secondary or even tertiary groups. When the protein molecule is split up by disrupting agents, including digestive ferments, the archon is set free to a greater or less extent. This archon is poison. Its poisonous character is due to its great affinity for the secondary groups. When the secondary groups are digested the archon aids the digestive enzymes in breaking down fresh protein molecules, and the digestion proceeds onward. The primary, or poison group causes the toxic symptoms of the infectious diseases. The non-poisonous or secondary group stimulates the

[1] "Protein Split Products in Relation to Immunity and Disease," by Victor C. Vaughan, M. D., LL.D., published by Lea and Febiger, Philadelphia and New York, 1913.

tissue cells to produce a special and specific proteolytic ferment or enzyme which has the power of rapidly digesting and destroying the homologous protein. Immunity to another attack of the disease is due to the rapid digestion of the invading bacteria upon subsequent exposure. The bacteria are thus destroyed before they have an opportunity to multiply.

Vaccines are prepared by attenuating by various means the disease-producing bacteria so that they are unable to cause infection, yet the bacterial protein still retains the power to produce the specific proteolytic ferment upon which immunity depends,—that is, according to the hypothesis of Vaughan. When a vaccine is used in the treatment of an infectious disease, this proteolytic ferment is produced in excess of the quantity required to digest the vaccine itself. and the excess is employed in digesting and destroying the invading pathogenic bacteria.

Various Teachings Compared with the Teachings of Vaughan.—We are taught by Metchnikoff that invading disease germs are destroyed by the white blood corpuscles or leucocytes, called by him phagocytes or "cell eaters" because they ingest and digest the bacterial cells. This does not conflict with that of Vaughan, for the phagocytes undoubtedly depend upon the ferments they contain for their proteolytic function.

How about the teachings of Ehrlich and his associates? They say that the destruction of invading bacteria is accomplished by the action of amboceptor and complement upon them, and that the value of bacterial vaccines lies in the fact that their injection into healthy tissues stimulates the tissue cells to produce a large excess of amboceptor which sensitizes the invading bacteria and thus prepares them for the destructive action of complement. *How does this view agree with that of Vaughan?* It is immediately apparent that the only difference is essentially one of terminology.

How do Vaughan's teachings agree with those of Wright, who says that the invading bacteria are destroyed by phagocytes only after they have first been prepared for ingestion by opsonins? It is evident that the opsonic properties of the serum are merely a manifestation of the action of the specific proteolytic ferment.

How does the explanation of the action of sensitized vaccine given by Garbat and Meyer and endorsed by Besredka, harmonize with that of Vaughan? This question is answered by Vaughan himself as follows: "Garbat and Meyer believe that immunization is secured only by the poisonous constituent, or constituents of the typhoid bacillus. According to my theory, the poisonous constituent of the typhoid bacillus has nothing to do with the production of immunity or sensitization. Sensitization and immunity are induced by the non-poisonous part of the typhoid bacillus."

The Relative Value of Living and Killed Sensitized Bacteria for Immunization.—In Besredka's earlier experiments killed bacteria were used. Further investigations, first on animals and then on man, demonstrated the harmlessness of certain sensitized living bacteria, and in anti-typhoid immunization the sensitized living bacteria are now preferred by Besredka and his followers.

Why are living bacteria preferred? It is generally conceded by authorities that immunity obtained by living viruses is more substantial than that resulting from

the use of killed virus. However, up to April, 1913, Broughton-Alcock has "seen no advantage of the living sensitized gonococci over those allowed to die."[2] Is there no danger of carrying contagion with living sensitized pathogenic bacteria? Besredka claims that there is no more danger of carrying contagion from living sensitized bacteria than there is from living smallpox vaccine, and that there is no danger from either.

Are sensitized bacterins prepared from killed cultures superior to unsensitized killed bacterins? Yes, according to Besredka, "Whatever be the nature of the virus, whether it be a question of bacteria of pest, of dysentery, of cholera, or of typhoid fever, whether it be a question of rabies virus, of diphtheria toxin, whether the bacteria are killed or living, sensitization confers upon them new properties which make them vaccines of the first order, and which are characterized by an action, *sure, rapid, harmless,* and *durable.*"

How do unsensitized living bacteria compare with unsensitized killed bacteria for the production of immunity against typhoid fever? Results obtained by Besredka and Metchnikoff in the immunization of chimpanzees against typhoid fever demonstrates that sensitized living germs gave absolute protection, causing but slight fever and reaction, while killed bacilla failed to protect adequately.

Is there no danger of acute infection from such procedure? Apparently the method is harmless, as Besredka reports that about ten thousand persons, men, women, and children, have been vaccinated without a single mishap of any kind.

Is there no danger that in some persons the injection of living bacilli might lead to the development of a carrier state? No cases of the kind have been reported in relation to the ten thousand persons immunized. Seven hundred of these cases were tested, and no typhoid bacilli found either in the feces or urine.

How do Besredka's results with living sensitized cultures compare with those obtained by the armies of the United States and of foreign countries with killed cultures? The results obtained by immunization with killed typhoid cultures in the armies of the world abundantly demonstrate that adequate protection against typhoid fever is obtained in this manner. However, according to Besredka's general verdict concerning the improvement in the vaccine produced by sensitization itself, killed sensitized vaccines are superior to killed unsensitized vaccines in all cases.

Sensitized vs. Unsensitized Bacterins. Is there anything to be gained by using sensitized killed bacteria for immunization? According to the statement by Besredka above quoted, there are many advantages to be gained by sensitizing the killed bacteria before injecting them for protective purposes.

What are the advantages? Freedom from the negative phase, freedom from local soreness and freedom from marked systemic reactions, and more rapidly acquired immunity.

What is meant by more rapidly acquired immunity? Experiments in the British army demonstrated that antibody formation following a dose of unsensitized killed bacteria administered for the prevention of typhoid fever, requires several days, and does not reach its maximum until the ninth or tenth day. Experiments

[2] "Vaccination for Various Infections with Living Micro-organisms," by W. Broughton-Alcock, Lancet, London, April 26, 1913.

by Besredka and his followers demonstrated that the maximum immunity following an injection of sensitized bacteria is reached in from twenty-four to forty-eight hours.

Dosage. What doses should be employed, and at what intervals should they be given, when killed sensitized bacteria are used for phophylaxis against typhoid fever? Want of experimental data on this subject makes of this a hypothetical question, requiring an answer of similar character. In the first place, if we accept Besredka's general verdict to the effect that sensitization confers upon bacterial vaccines certain properties which markedly increase their efficacy, whether prepared from living or killed cultures, we must apply the verdict to killed sensitized typhoid bacilli. Secondly, the doses successfully employed by Besredka's associates in treating typhoid fever are very much larger than the doses recommended by Wright and his followers for therapeutic use, when killed cultures are employed. Broughton-Alcock's impression from experience is that great value lies in large doses. He begins with 500 to 3000 million killed gonococci, and has also found it to be a safe and beneficial procedure to commence with 20 million killed staphylococci and 200 million killed gonococci.

What dosage should be employed in the treatment of infectious diseases? Gordon reported excellent results from the use of killed sensitized strepto-bacterin in a series of cases treated at St. Bartholomew's Hospital. He employed the "intensive" method, which consists of giving rapidly increasing doses at brief intervals. The dosage in the four following cases illustrates his method:

In Case 1—(A girl suffering from erysipelas) he administered 100 million sensitized streptococcus vaccine as the initial dose, 24 hours later 500 million, and on the following morning 1000 million. Three days later the child was quite well.

In Case 2—(A nurse with cellulitis of the scalp and cervical adenitis), the patient was given an initial dose of 100 million, the following day 500 million, and 24 hours later 1000 million. Two days later the erysipelas subsided, the temperature fell, and convalescence ensued.

In Case 3—(A patient suffering with an acute attack of erysipelas of the forehead and cheeks), three successive doses of 100, 500 and 1000 million sensitized streptococcic vaccine were given at 24-hour intervals, followed by a prompt cure.

In Case 4—(A patient who suffered from a compound fracture of the lower end of the left humerus which had suppurated, and cultures from which showed streptococcus pyogenes), a single dose of 100 million (this may be a typographical error; possibly 1000 million is meant) sensitized streptococcus vaccine was given. The local condition improved and the pyrexia subsided.

The dosage employed by Boinet in treating typhoid fever illustrates another system of dosage. He says: "The best results follow the use of doses of 2 cc., repeated daily during four consecutive days, if the disease is grave, but three consecutive days if the disease is mild." He used the living sensitized germs.

In reporting cases, other observers, while careful to state intervals, neglect to inform us as to the size of the doses. Broughton-Alcock reports cases of gonorrhea, acne, sycosis, furunculosis, impetigo, and seborrheic eczema, treated with appropriate sensitized living bacteria. In cases of acute and chronic gonorrhea without complications, the serobacterin was apparently without benefit. In cases of orchitis, epididymitis, and in gonorrheal arthritis and periarthritis, good results were invariably secured. In every case following the second injection, and in many cases following the first injection, the pain ceased and the swelling was notably diminished. The arthritis and periarthritis were arrested. To avoid

relapse, the injections were repeated. It is important to know the size of doses and intervals to give these reports proper educational value.

Summary of Besredka's Claims in Regard to Sensitized Bacterins.—The most striking characteristics of sensitized vaccines are:

1. They produce but slight local reaction (inflammation at site of injection),
2. They cause no general reaction (malaise, increased temperature, etc.),
3. They may be given in much larger doses and much more frequently than the unsensitized bacterins (every 24 hours),
4. The immunizing effect is almost immediate (manifesting itself within from 24 to 48 hours),
5. They sometimes give successful results in very late stages of a disease, when no response is secured from the ordinary bacterins, and even serum treatment is ineffective."[3]

According to Besredka, "whatever be the nature of the virus, whether it be a question of bacteria, of pest, of dysentery, of cholera, or of typhoid fever, whether it be a question of rabies virus, or diptheria toxin, whether the bacteria are *killed* or *living,* sensitization confers upon them new properties which make of them vaccines of the first order, and which are summed up in an action *sure, rapid, harmless* and *durable.*"

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WHAT SOME PHARMACISTS ARE FIT FOR.

F. A. BONGARTZ, MEMBER NEW JERSEY BOARD OF PHARMACY.

Since I have had to rate about one hundred and sixty papers of our last and my first State Board examination, and in view of the low standard of same, I think it would be well to enact a law disbarring about 33 1-3 percent of pharmacists from selling anything but shoe strings and postage stamps.

We should raise the standard, then protect the standard and give the public the benefit of the raise.—*N. A. R. D. Journal.*

[3]Bull. de L'Inst. Past., Tome VIII, 1910, 30 Mars, 6 PP. 241-253.