Scientific Section

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PRINCIPLES UNDERLYING THE USE OF VACCINES, BACTERINS, ANTITOXINS AND IMMUNE SERUMS AS AGENTS FOR THE PREVENTION AND CURE OF INFECTIOUS DISEASES.*

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This is the first of a series of papers. The second will deal with Diagnostic Reagents and their uses in the diagnosis of infectious diseases. The third will consider the application of biologic products as therapeutic agents.—[EDITOR].

Infectious diseases are caused by the growth and multiplication in the body of bacteria, protozoa, moulds and yeasts. They are combats between lower and higher forms of life according to the general law of struggle for existence constantly going on between all forms of living matter. Closer analysis shows that the battle is between the protoplasm of the conflicting forces, with enzymes as weapons of offense and defense.

Groups of symptoms and phenomena caused by the growth and multiplication of these living organisms and representing the external or visible evidences of their growth and multiplication in the body have, in the past, been considered as diseases, in themselves. These phenomena may be compared to the noise and smoke of battle; the real conflict being between the microbial cells and the body cells. In the prevention and treatment of infectious diseases, we aim our weapons at the combatants, not at the noise and smoke.

The phenomena of recovery from infectious diseases have been investigated and as a result we have a new therapeutics in which modified infective agents and products resulting from artificially immunizing animals are used in treatment.

Bacteria. These one-celled microörganisms, now so well known to bacteriologists, are classed by some authors with the vegetables, others class them with the animals. However, their classification is more of an academic question than one of practical importance. The bacterial cell may be berry-shaped, rod-shaped, or spiral, giving rise to the names coccus (pl. cocci), bacillus (pl. bacilli), and spirillum (pl. spirilla), respectively. The cocci may grow in pairs (diplococci), in bunches like grapes (staphylococci), in chains (streptococci), etc. And their

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[1] *Bacterial Diseases*:—Diphtheria, pneumonia, influenza, whooping cough, gonorrhea, etc. *Protozoan Diseases*:—Malaria, syphilis, relasping fever, sleeping sickness, etc. *Mould Diseases*:—Aphthae or thrush, pityriasis versicolor, etc. *Yeast Diseases*:—As incitants of disease in man yeasts have been much studied since 1894. Prominent in the literature are the contributions of Busse, Gilchrist, Curtis, Ophüls and Zinsser.

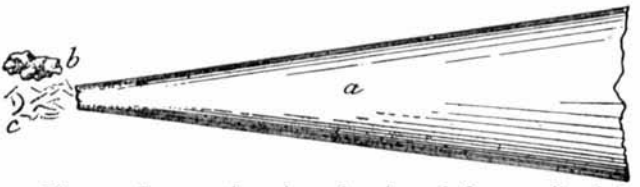


Fig. 1.—Comparative size of point of fine needle (a); bit of dust (b); bacteria (c).

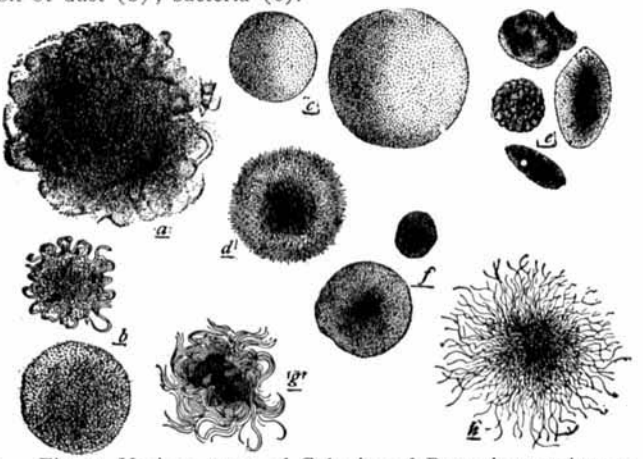


Fig. 2—Various types of Colonies of Bacteria growing on gelatin.

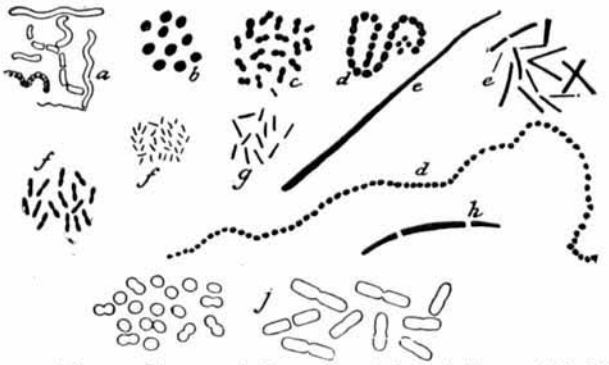
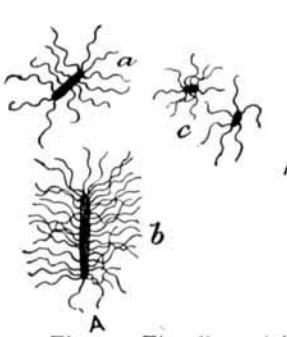


Fig. 3—Shapes of Bacteria. (a) Spirillum; (b) Micrococcus; (c) Diplococcus; (d) Streptococcus; (e-h) rod-shaped bacteria; (i and j) Divisions.



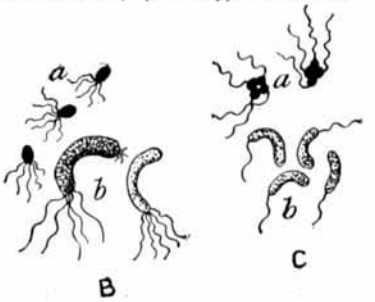


Fig. 4—Flagella. (a) Perutruchic; (b) Lophotrichic; (c) Montrichic.

Fig. 5—Spore Production.

Illustrations from Conn's "Practical Dairy Bacteriology," published by Orange-Judd Co.

cultures may be colored giving such names as aureus, citreus, albus, etc. Their size, rapidity of growth, method of reproduction, sporulation, etc., are important subjects in relation to their method of growth and multiplication in the body as infective agents, but the limits of this paper will not permit detailed discussion and the reader is, therefore, referred to works on bacteriology.

Protozoa. The protozoa are essentially unicellular (one-celled) animals. The individual or "person" in this grade of the animal kingdom is a single cell; and, although we find protozoa which consist of aggregates of cells, yet an examination of the details of their structure and life history establishes the fact that the cohesion of the cells in these aggregates is not an essential feature of the life of the individual protozoon but a secondary and non-essential arrangement.

Yeasts and Moulds. The yeasts or blastomycetes and the moulds or hyphomycetes are closely related organisms, distinguished from each other and from the bacteria by morphological characteristics and methods of growth. The limits of this paper will not permit a detailed description. The vital properties or "functions" of living protoplasm in so far as this study is concerned, are best exhibited by the living protoplasm of the amebae.[2]

The Ameba. The ameba is a protozoan having a simple protoplasmic body with a nucleus and neucleolus and granules. The constant flowing out or extension of an ameba or other mass of protoplasm into irregular processes, called pseudopodia and their subsequent retraction or effacement, is termed *ameboid movement.* In the higher plants and animals the movements of the protoplasm are combined and directed so as to produce effects in relation to the whole organism built up of countless cells. The white corpuscles or leucocytes appear to be masses of free formative protoplasm, having the power of locomotion (ameboid movements) and capable of assuming various shapes.

Under the microscope a pseudopodium is observed to extend and into it the granules stream in constant current until the whole protoplasmic body has changed its location and form. These movements, ameboid movements and granule streaming as manifested by the protozoa, are intimately related to the processes of hunting, seizing, and ingesting food, and of the intercourse of the individuals of a species with one another and their evasion of hostile agencies. The same applies to the movement of cilia and flagella, which subserve the needs of the individual cell of which the moving protoplasm is the main substance.

Plants, Animals and Man.—In a Sense Colonies of Amebae. Plants and animals (including man), while made up of special organs and tissues and cells, yet consist of cell-units which manifest vital phenomena or functions inherent in the protoplasm itself, namely, contractility, irritability and automatism, reception and assimilation of food, metabolism with secretion and excretion, respiration and reproduction, phenomena we accordingly recognize as muscular or nervous, secretory or excretory, respiratory, reproductive and the like.

Yet in these organs and their tissue cells, however specialized to one function only, a residue of all or nearly all the other fundamental properties of protoplasm

[2]The ameba and the foraminfera afford convenient and classical examples of the protoplasm of the lowest animal forms; the white corpuscles of the blood, or leucocytes should also be examined. Vegetable examples are readily obtained from the cells of a growing shoot; while the living cells of *chara*, a genus of cryptogams, and other examples of protoplastic movement, should be observed.

remains and may be developed; and thus those changes which we call "adaptation to environment," and those pathological disturbances we term "disease" are alike provided for.

We thus find the vital functions of protoplasm taking part in the warfare between infective organisms and body cells.

The intracellular digestion of food by these lower forms of life has been contrasted with the "cavitary" or entral digestion of higher animals, that is, the digestion that goes on in the alimentary canal. In the latter, enzyme and acid (or alkalies) are poured out by the cells of the secreting organs and glands into the digestive canal, and digestion is extra-cellular.

The digestive organs and tissues of the higher animals are specialized. The digestive glands of the mouth produce ptyalin, a starch digester; the gastric glands produce pepsin, a protein digester; the pancreas furnish a digestive "juice" containing three enzymes, trypsin, amylopsin, and steapsin, which respectively digest proteins and starch, and split up fats.

The enzymes produced by the protoplasm of bacteria and protozoa are not the products of specialized organs and tissues as is the case with the higher forms of animals, but they are capable of digesting the proteins and carbohydrates which constitute their food. In the same manner the cells of the body are able to digest substances with which they are brought in contact.

The digestion carried on by the body cells not directly related to the alimentary canal and its secretory organs is known as *parenteral* digestion. For example, when protein, living or dead, is introduced into the tissues of an animal or man, the body-cells are stimulated to produce a specific proteolytic enzyme or digestive ferment which digests and destroys it.

What Is Meant by Pathogenicity. The protoplasm of the infective agent, having become a parasite, lives at the expense of the tissues of the host. As stated by Vaughan, the pathogenicity of a bacterium, or its power of producing disease, is dependent upon its ability to grow and multiply in the body tissues. Any microörganism which can grow and multiply in an animal body is pathogenic to that animal. To grow and multiply in the animal body, the invader must convert the proteins of the animal into its own proteins. A foreign protoplasm (bacteria, protozoa, etc.), can grow and multiply in the human body only if the invader is capable of digesting and utilizing the proteins of the body. All living cells grow by means of their own digestive ferments, and these must act upon the pabulum within their reach. If the ferment of the bacterial cell cannot digest and prepare food for the bacterium from the body protein (protoplasm) then the invading bacterial cell dies. If the digestive ferment produced by the body cells is rapidly and thoroughly destructive there is no bacterial development and the organism is innocuous.

Structure of the Protein Molecule. Protoplasm is composed chiefly of protein. According to Vaughan and his co-workers, all true proteins are constructed upon the same general plan, and consist of a central group, keystone or archon, around which are arranged sub-groups. The central group is common alike to all proteins. It is a poison, but not a toxin, that is, it is incapable of stimulating the body cells to produce an antitoxin when introduced into the animal body. Its poisonous properties are due to its powerful affinity for the secondary groups

of other proteins. The secondary group or groups of each protein molecule is specific. The power of the protein to stimulate the body cells to produce a specific proteolytic enzyme resides in the secondary groups.

Typhoid Fever as an Illustration of Infection and Subsequent Immunity[3]. The infective agent of typhoid fever is the typhoid bacillus. It is infective because by means of its digestive ferment it can feed on the proteins of man's body. This means that it can convert man's proteins into typhoid proteins and thus multiply its kind. Moreover, it is not, immediately on its entrance in man's body, destroyed by the ferments of the body cells. Having found admission to the body it proceeds to grow and multiply. This continues through the period of incubation, which in this disease is somewhere about ten days. During this period of incubation there is no effective resistance on the part of the body cells to the growth and multiplication of the foreign protein. During this time the man is not sick, and we conclude therefore that it is not the growth of the foreign protein which *per se* gives rise to the symptoms of typhoid fever. However, during this time the body cells are being prepared for their combat with the foreign protein. This preparation consists of the development in certain of the body cells of a new function, that of elaborating a new and specific ferment which will digest and destroy the foreign protein. When this new ferment begins its action the first symptoms of the disease appear. The active stage of the disease, with its symptoms and the lesions induced, marks the period over which the parenteral digestion of the foreign protein exists. Death may come from the too rapid breaking up of the foreign protein and the consequent liberation of a fatal dose of the protein poison, which is always formed on the disruption of the protein molecule, or it may result from some lesion induced by the products of this disruption, such as perforation and hemorrhage, or it may follow from chronic intoxication and consequent exhaustion. In case of recovery the individual is, for a time at least, immune to the typhoid bacillus because his body cells are now able to elaborate and make immediately effective the specific ferment which destroys the typhoid protein.

To this stimulating action of the secondary group of the protein molecule upon the body cells which causes the development in the body of the man a specific proteolytic enzyme, Vaughan has given the name "protein sensitization." "There is developed in certain body cells a new function, that of elaborating this new ferment."

Phagocytosis. Metchnikoff has demonstrated that those body cells, known as leucocytes, and commonly called white corpuscles of the blood, possess the power of digesting and disposing of foreign proteins when introduced into the animal body. The leucocytes are little masses of free protoplasm, similar to the ameba and capable of ameboid movement. The leucocyte, like the ameba, in feeding, projects a pseudopodium outward until it comes in contact with the food particle, which it now proceeds to engulf, and by means of its enzymes, digest. Metchnikoff teaches that the leucocytes have the power of destroying bacteria invading the body, and on that account gave to these organisms the name "phagocytes," or "cell eaters" (from *phagein,* to eat and *kutos, cell*). It is now generally conceded

[3]This illustration is taken from Vaughan's book entitled "Protein Split Products in Relation to Immunity and Disease."

that the tissue cells, as well as the leucocytes, possess the power of phagocytosis. They have, therefore, been fancifully described as "sessile phagocytes."

According to Metchnikoff, the digestive power of the phagocytes is exerted through the agency of enzymes or ferments known as "cytases."

FUNCTION OF THE BODY FLUIDS IN IMMUNITY.

Bacteria may be destroyed by the digestive action of the body fluids into which the enzymes have been discharged, and which therefore possess the same power of destroying bacteria originally characterizing the phagocytes themselves. Serum from the blood of an immunized animal (immune serum) manifests three main specific actions, namely, (a) bactericidal and lysogenic action; (b) opsonic action; (c) agglutination and the closely allied precipitating action. It is, therefore, assumed that these actions are due to certain antibodies. The bactericidal and lysogenic action is assumed to be due to the presence of *bacteriolysin* (*lysis,* to dissolve). Some authors believe that the body fluids also contain *bactericidin,* having the power of killing bacteria without dissolving them. The opsonic action is assumed to be due to *opsonins.* When a small quantity of immune serum is added to a suspension of the corresponding bacterium, the organism becomes agglutinated into clumps and motility is suspended or destroyed. This action is assumed to be due to the presence of *agglutinins.* The immune serum may not only cause agglutination, but when added to the filtrate of a culture of the corresponding bacterium, may produce a cloudiness and afterwards a precipitate. The name *precipitins* has been given to these hypothetical substances.

Antigens and Antibodies. In overcoming the infective agent the body cells of the animal undergoing the immunizing process develops certain protective substances above referred to. Possessed of these protective substances, the animal can subsequently withstand a more severe attack of the same infection and is, therefore, said to be immune. These protective substances are classed under the general head of antibodies, and the substances used for immunization are called *antigens.*

Practically any protein substance may serve as an antigen. Its injection gives rise to specific proteolytic, or digestive ferments, as we have already seen. According to Vaughan, all enzymes are composed of *complement* and *amboceptor.* The specific part of enzyme is the amboceptor. Each kind of protein when introduced into the animal body stimulates the body cells to produce specific amboceptor, the function of which is to prepare the protein for the lytic action of the complement. As already stated, the complement is not specific. It is not increased by the immunizing process. It is practically the same no matter from what animal obtained. It will cause the lysis of any kind of protein, provided the latter is first prepared for lysis by union with its appropriate specific amboceptor. For instance, if egg albumen is used as an antigen, the body cells are stimulated to produce specific proteolytic amboceptor capable of *sensitizing* egg albumin. The same applies to serum albumins and globulins, milk, epithelial cells, vegetable albumin, etc. It also applies to bacteria. Each kind of bacteria requires a specific amboceptor for its digestion. This amboceptor must be produced by the body cells and unite with the antigen (bacterium) before the complement can act upon it and cause *bacteriolysis.*

Stimulins, Opsonins and Bacteriotropins. It has long been taught by Metchnikoff and others that there are certain substances in the body fluids which aid phagocytosis. Metchnikoff regarded them as *stimulins,* or substances that stimulate leucocytes to become more actively phagocytic. On the other hand, Wright, Douglas, Hektoen and others have demonstrated that these substances aid phagocytosis, not by stimulating the leucocytes, but by preparing the bacteria for ingestion and digestion by them. Wright gave the name opsonins to these substances (from the Greek word *posono,* "to prepare food for."). They are also known as *bacteriotropins.* The source of opsonin is unknown. Thermostabile opsonin (opsonin not affected by low heat, 56-58° C.) is increased by artificial immunization and by disease. It is largely of the nature of amboceptor, which is also thermostabile, and increased by the immunizing process. It is, like amboceptor, specific, and is probably produced by the body cells, especially by the local cells at the site of infection. Thermolabile opsonin—largely occurring in normal serum—is, like complement, non-specific, and is not increased by the process of immunization. Complement is also thermolabile.

ACTIVE IMMUNIZATION.

The process of stimulating the body cells to produce antibodies (enzymes) is called *active immunization.* The immunity following disease is an example, but the term is usually applied to artificial immunization.

Vaccination. A vaccine is an infective agent so modified as to prevent its growing and multiplying in the body, but not so changed as to destroy its power as an antigen. When a vaccine is introduced into the body in small doses the body cells react, producing antibodies (enzymes), and a resulting immunity against the corresponding infectious disease, without marked disturbance to the system or danger to life.

Smallpox Vaccine. This is an attenuated living virus consisting of the infective agent of smallpox modified by passage through the cow. The virus in its passage through the cow becomes so modified that it can no longer produce smallpox, but is still able to stimulate the production of the specific antibodies (ferment, or specific amboceptor) against this disease. Centuries before the Christian era the Chinese observed the immunity against a second attack enjoyed by those who had survived smallpox. By inoculation, i. e., artificial transfer of the smallpox virus, they endeavored to check the spread of the disease. The people of eastern countries were also accustomed to expose their children to a mild case of smallpox in order that a similar mild attack might produce immunity against the disease. These practices, however, were not without risk, as this mild disease not infrequently became a virulent one, and it was also found that the disease so produced could be spread as readily as the natural form. It was long known that milkmaids who contracted smallpox from sores on the udder of the cow were immune against smallpox. Edward Jenner in 1796 demonstrated experimentally that when the virus of cowpox is applied to the abraded skin of a human being a trivial affection, since called "vaccinia," results and that this is followed by complete or almost complete immunity to smallpox for a long period of years. Re-vaccination should be practiced after five to seven years to insure a more potent and lasting immunity.

Rabies Vaccine. The work of Jenner was purely empirical. Pasteur laid the foundation of scientific immunology in 1858, 1860, 1863. It was not until 1879 that he made the discovery that light, high and low temperature, and exposure, could so reduce the virulence of an infective agent that while its injection into an animal was practically without danger or ill effect, it could stimulate the mechanism of immunity in the host and produce a lasting protection against infection.

This fact was accidentally discovered by Pasteur when working on chicken cholera. He found on returning from an absence from his laboratory that the cultures of the chicken cholera organism with which he had been experimenting had become innocuous. The far reaching importance of this fact at once impressed him and resulted in his demonstration that a mild form of chicken cholera may be produced by using as antigen an attenuated organism, and that this mild attack would confer immunity against the severe form of the disease. On this discovery is based modern bacterin and serum therapy.

Shortly after this epoch-making discovery Pasteur (1880) startled the world by announcing that rabies resulting from mad dog bite could be prevented by vaccination. The process consists of active immunization with emulsion of the spinal cord of rabid animals (rabbits) reduced in virulence by drying for a certain length of time over caustic potash. The incubation period in rabies is comparatively long, varying from three weeks to perhaps several years, while that of the attenuated virus is relatively short, thus making it possible to administer effective vaccination after the infection of the patient by the bite of the rabid animal.

Bacterial Vaccines or Bacterins. These antigens consist of suspensions or so-called emulsions of pathogenic bacteria, modified by heating them to a temperature sufficient to destroy their viability or securing the same results by antiseptics, but not so altering them as to destroy their power of stimulating the body cells of the vaccinated individual to produce antibodies. The suspension is usually made in physiological salt solution, which should be isotonic with the blood, and they are standardized either by bacterial count or by determining the amount of bacterial products in each cubic centimeter by drying and weighing. They are administered by subcutaneous injection or intravenously, and used either for prophylaxis (antityphoid immunization), or for treatment. The blood serum of the individual subjected to a dose of bacterial vaccine manifests increased opsonic power, that is, power to prepare bacteria, of the same kind injected for ingestion and digestion by the phagocytes.

Stensitized Bacterial Vaccines. When pathogenic bacteria are treated with immune homologous serum, i. e., serum from the blood of an animal immunized against the same kind of bacteria to be used for preparing the vaccine, their viability is so reduced that they become incapable of reproduction yet still potent as antigens. Such bacteria are described as "sensitized." By this method the specific amboceptors contained in the immune serum are made to combine with the bacteria, thus preparing them for the immediate action of complement when introduced into the body of the person to be immunized. The blood serum of the individual subjected to a dose of sensitized bacterial vaccine manifests increased bacteriolytic power. According to Garbat immunity following the injection of

sensitized bacterial vaccine is largely due to increase in "bacteriolysin" rather than to increase in "opsonin."

Sensitized Killed Bacterial Cultures. These products differ from sensitized living bacterial cultures in being absolutely safe in so far as *viability* is concerned. It is believed by some that the use of living sensitized cultures may possibly cause carriers and spread disease. Until this and other important questions are answered in a satisfactory manner the U. S. Government will continue to refuse license for their commercial manufacture.

In explaining the action of sensitized vaccines, Besredka refers to the researches of Garbat and Meyer. These investigators aver 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 (endotoxin) set free. Both portions are toxic; both give rise to individual immunizing substances by stimulating the tissue cells to produce them. Immunity is, therefore, partly due to the production of endotoxin and partly due to the production of bacteriolysin.

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 viewpoint. He replied as follows:

UNIVERSITY OF MICHIGAN, ANN ARBOR, Nov. 8, 1915.

Dear Doctor Stewart:

It seems to me that the action of sensitized bacteria compared with the 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 nonpoisonous 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 greater jeopardy. Garbat and Meyer believe that immunization is secured 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 nonpoisonous part of the typhoid bacillus. Subjecting the typhoid bacillus in vitro to immune serums, 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 Freidberger, 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 nonpoisonous part of typhoid bacteria. This nonpoisonous part which I have obtained has been secured by a crude way. The nonpoisonous 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 nonpoisonous 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.

V. C. VAUGHAN.

ADVANTAGES OF SENSITIZED OVER NONSENSITIZED BACTERINS.

The following advantages of sensitized over nonsensitized bacterins are noted:

1. As a rule they produce but slight local reaction (inflammation at site of injection). This rule has its exceptions. Occasionally severe local reactions follow their injection. However, this is not as likely to occur when using the sensitized bacterin as when the nonsensitized are employed.

2. They cause no general reaction. This is the rule, but it has exceptions. General reactions are manifested by malaise, increased temperature, etc.

3. They may be given more frequently and in much larger doses than the unsensitized bacterins (every twenty-four to forty-eight hours). Occasionally persons are found who cannot digest larger doses. In such cases the dose should be decreased.

4. The immunizing effect is almost immediate (manifesting itself within from 24 to 48 hours), instead of 9 to 10 days.

5. The course of treatment for immunizing an individual against typhoid fever used by the U. S. Army is to inject 500,000,000 killed typhoid baccilli subcutaneously, to be followed 10 days later by the injection of the second dose (10 million killed bacteria), and after another interval of 10 days by the injection of the third dose (1000 million killed bacteria). The dosage of sensitized typho-bacterin is twice as large, i. e., 1st dose 1000 million, 2d dose 2000 million, 3d dose 2000 million. Intervals 6 to 7 days. In the event of a threatened epidemic the advantages in favor of a quick acting bacterin immunity against typhoid fever are not difficult to realize.

6. According to Besredka, sensitized bacteria give results in very late stages of the disease, when no response is secured from the ordinary bacterins, and even serum treatment is ineffective.[4]

7. Owing to the fact that the specific bacteriolytic amboceptor is already united to the antigen (bacteria), and therefore ready for prompt digestion by the body cells when injected it follows that the body cells are saved from the

[4]Bulletin de l'institut Pasteur, viii, 6, pp. 241-253, Mars, 1910.

effort of producing amboceptor, an effort which the body cells of a patient sick with typhoid fever may be unable to accomplish.

8. There is not the same liability of producing what is called a "negative phase"—the temporary condition sometimes following the injection of an ordinary bacterin owing to the using up of the normal opsonin in the patient's blood, the same being required to prepare the injected bacterin for phagocytosis.

PASSIVE IMMUNITY-SERUM THERAPY.

So called because the body cells of the individual undergoing immunization have no part in producing it. Active immunity is produced in some other animal, usually the horse, and the individual to be passively immunized acquires immunity by receiving injections of immune serum taken from the immunized animal. The use of diphtheria antitoxin to prevent diphtheria (prophylactic immunization), and as a curative agent in its treatment (curative immunization) illustrates the main purposes for which passive immunization is employed.

Although sufficient evidence has not yet accumulated to permit positive statements, it is believed that more than one antibody is usually present in each immune serum, and that the immunity acquired by their employment is due to several antibodies, which, acting together, tend to overcome infection.

As stated by Kolmer[5], "From the practical standpoint, therefore, immune serums may be used to produce two main types of passive immunization, namely:

"1. *Antitoxic immunization,* due to antitoxins opposing the true extra-cellular toxins, as in diphtheria and tetanus (antitoxic immunity).

"2. *Antibacterial immunization,* due mainly to bacteriolysins and bacteriotropins (antibacterial immunity)." Serums containing these antibodies are useful in the treatment of infections due to the meningococcus, pneumococcus, streptococcus, gonococcus, etc.

I am forced to end this paper abruptly to permit its publication in the JOURNAL. It is my intent to continue the subject in subsequent papers.

STABILITY OF PREPARATIONS CONTAINING YELLOW PHOSPHORUS.*

H. ENGELHARDT AND O. E. WINTERS.

In our previous paper entitled the "Estimation of Yellow Phosphorus"[1] read at the Detroit meeting we announced our intention to substitute other copper salts for copper nitrate, which we used in the process suggested.

The tabulated results of this work show quite plainly that no advantage is gained by the use of other salts of copper over the nitrate.

With the exception of those made with copper chloride, the results obtained when employing other copper salts were fairly uniform. Copper chloride is the least appropriate for this purpose, due to the apparent decomposition of the

[5] A Practical Text-book of Infection, Immunity and Specific Therapy, by John Kolmer, M.D., Dr. P.H., Phila. W. B. Saunders Co., 1915.

* Read in Scientific Section A. Ph. A., San Francisco meeting.

[1] Journal A. Ph. A., Apr. 1915, p. 451.