NOTE TO USERS

This reproduction is the best copy available.

UNIVERSITY OF CINCINNATI

June 2 1930

I hereby recommend that the thesis prepared under my supervision by Ethel Lenna Hoppham

entitled The Study of Dermal Reactions in the Selection of Bacterial Antigens in Biological Therapy

be accepted as fulfilling this part of the requirements for the degree of Philosophy

Approved by:

C. A. Mills

Stanley Dorst.

Mark A. Brown

Form 668—G. S. and Ed.—500—3-30.

The Study of Dermal Reactions in the Selection of Bacterial Antigens in Biological Therapy.

A dissertation submitted in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

to the Graduate School of the University of Cincinnati

1930

by

Ethel Linna Hopphan

B. S. Michigan State College, 1919

Table of Contents.

I. INTRODUCTION.

Biological therapy today occupies a curious position in the field of medical therapeutics. Forty years ago it was acclaimed as the culminating achievement of a century during which the art of medicine stood aside and watched science advance with a rush of accomplishment which forced the labor of many past centuries into the misty shadows of metaphysics. It was especially that part of biological therapy which dealt with the use of vaccines both in the prevention and cure of disease which appeared to hold the most important possibilities in the conquest of disease.

After thirty years what has come of those bright visions? We ask the practical man of medicine what he thinks of vaccine therapy and we find that he thinks very little of it if, indeed, he thinks of it at all. Much of biological therapy, we find, has now taken its place in that limbo of misty shadows and vaccines, so it would seem, have been the creation of avaricious commercial houses, invented to delude the honest practicioner of the art of medicine. If we hesitate to accept this evaluation and timidly ask concerning those outstanding instances of

biological therapy which are a part of sound medical treatment we are likely to be told that these represent the little good which has grown out of great evil and ce certainly there is "nothing in vaccine treatment". Our hypothetical medical man will, if he runs true to his guild, become quite bitter in his insistance upon the futility of such therapy for indeed he has given ———No. 28 or ————No. 9, according to directions and nothing good has ever come of it.

It must be said in defense of this attitude that there is sound reason for its existence and the clinician may well look with disfavor upon his colleague who is given to the indiscriminate use of vaccines in treating his patients.

We felt that an adequate study of vaccine therapy was indicated before the prevailing views could be accepted. Such a study should be undertaken from the clinical as well as the laboratory viewpoint and should follow the patients thru months and years, not weeks. It was to this end that the studies outlined in this thesis were undertaken and carried thru. We have preferred to study a relatively small group of patients carefully and over long periods of time, believing that it is better to know accurately the story of one man than to draw conclusions from the general drift indicated by opinions concerning many.

If, in the course of this work we have revived somewhat a dying interest in what we believe to be a most important form of biological therapy, well and good; but we are more definitely interested in establishing a rational method of approach which shall, we hope, act as a point of departure for future investigation.

So great has been the confusion which has arisen in these early days of experiment and treatment that a somewhat detailed review of historical developments covering the period between 1900 and the present is essential as a background for our work.

Historical

The practical use of bacterial vaccines is acknowledged to be an outgrowth of careful investigational work done by Sir A. E. Wright.[1] He had become interested in Metchnikoff's phagocytic theory [2] which was originally formulated in 1883 in this manner: the leukocytes and certain other cells may be regarded as specific fighting cells able to engulf and consume living as well as dead bacteria and cellular debris. According to Metchnikoff, the outcome of any infection depended upon the success or failure of the phagocytes to overcome the invader. He believed that phagocytosis is stimulated by the action of certain substances contained in immune serum upon the phagocyte, itself, thereby increasing its power to ingest

and to digest the invading organism. Denys and Leclef [3] opposed this view of Metchnikoff, attributing the increased phagocytosis to the action of an immune serum upon the bacteria operating in some way so that they were more easily ingested by the phagocytes.

Wright first determined the direct dependence of phagocytosis upon some substance in the immune serum. He proved that the serum component acted upon the bacteria directly, was bound by the bacteria, and rendered them subject to phagocytosis. This substance was thermolabile, being destroyed by heating to 60° C. for ten or fifteen minutes; it was therefore, a new substance and was termed by Wright an opsonin meaning "to prepare food". Furthermore, Wright showed that in certain cases, particularly staphylococcus, typhoid and tubercle bacillus infections, the phagocytic powers of the individual were relatively diminished toward the infecting organism, but could be increased, specifically, by active immunization with dead bacteria.

Much of Wright's early work was directed toward the control of typhoid fever among the British soldiers in the British Colonies. The first bacterial vaccines used by Wright, about 1900, consisted of four week old broth cultures of virulent typhoid bacilli sterilized by heating at 60° C. and preserved with one per cent of

lysol. These vaccines were standardized against guinea-pigs. Of course such preparations were extremely toxic, bringing about severe general as well as local reactions even when given in small quantities. The loss of time by the soldiers following these injections was no less than that resulting from typhoid fever, itself.

The next step in the development of vaccine therapy was the use of suspensions of twenty-four hour cultures grown upon nutrient agar slants at 37° C. in physiological sodium chloride solution, 0.85 per cent, and heated to 60° C. or higher. These proved less toxic and consequently could be tolerated in much larger quantities. The reactions in man, however, were still fairly severe.

From statistics sent to him by the various divisions of the Army, Wright concluded that such an injection of dead typhoid bacilli conferred upon the recipient a certain measure of protection against typhoid fever; a second injection afforded added protection. Among the ones inoculated who developed typhoid fever subsequently, the course of the disease was a much milder one. The incidence and death rates were diminished; the protection persisted for a minimum of three years.

In regard to the reactions obtained, it may be pointed out that Wright advised treatment with a small dose to be repeated after a short interval of time. With smaller doses the symptoms were limited to a slight

tenderness at the site of the inoculation and a mild malaise. The reactions varied with the individual, the dose being identical. Much benefit can be derived from a careful examination of those reports of Wright's early work. Interpret the findings as you will, many of his practices may still be followed.

By 1902 Wright was extending the treatment with bacterial vaccines to include the use of them therapeutically, in cases of localized bacterial invasions such as furunculosis, sycosis and acne, by the injection of staphylococcus vaccine.

Meanwhile Wright was much interested in his opsonic index determinations. From observations of his results he found that following an injection there was a period during which the individual had a lowered opsonic index; this period was in turn followed by a second period when the opsonic index increased above the normal, indicating to Wright, increased resistance. Later the opsonic index returned to nearly the initial index, being, perhaps, slightly higher. This increase above the initial index seemed to be cumulative so that eventually the phagocytic power could be very definitely increased and maintained at a higher level for approximately a year. This is termed the theory of the ebb, the flow, and the return. The ebb was also termed the negative phase; and the flow, the positive phase. Wright found that the negative phase might

be suppressed if the dose were sufficiently small to produce no constitutional disturbance; it might though, be exaggerated and prolonged, were the dose large enough to produce a general reaction. In one case he was able to avoid the negative phase entirely. In all cases there was a certain tendency to relapse after some time.

Various methods of standardization were evolved and discarded. The first method, as mentioned above, being standardization against guinea-pigs; the second was by measurement of amount of surface growth injected; the third and last method, which was used for many years was a method of counting the number of organisms by comparing them to normal red blood cells in a stained smear. By trial and error he found what he considered a proper quantity by number of each individual organism to be injected.

Thus Wright finally advocated the standardization of vaccines by counting, and the regulation of dosage by the opsonic index determination. The initial dose should be small enough not to exaggerate the negative phase, and subsequent inoculations should be made in the positive phase. Extension of this treatment to include such diseases as bronchitis but not tuberculosis nor generalized infections was advocated.

Wright's reports in the literature are numerous and judging by the quantity and variety of the literature

upon this subject, he seems to have instigated considerable investigation on the part of many others. However, most of this work was merely confirmatory and offered nothing particularly new. Such a report is the one of Bulloch in 1905 [4], who reviews the work and theories of Wright and summarizes the consensus of opinion at that time: "The therapeutic value of such vaccine treatment, as claimed by Wright, is easy of demonstration. The rational explanation of the results is not so clear. It is an apparent paradox that an individual may suffer from a staphylococcus furunculosis for months, and yet the introduction, into his subcutaneous tissue, of a small quantity of the very coccus which has been the cause of his trouble, may bring about a rapid disappearance of already existing boils, and a prevention of others which, according to all clinical evidence, would have occurred".

Bulloch found that more inoculations were required to effect a cure than had Wright.

Stock Vaccines.

The use of stock vaccines apparently originated in St. Mary's Hospital, London, the institution with which Wright had previously been connected. In 1908 Mathews [5], of that hospital, suggested that for practical purposes too much time was involved in the determination

of opsonic indices. For that reason he had dispensed with them and was using stock vaccines, either of his own preparation, or from a commercial house. He still felt that vaccines were best used when controlled by the opsonic indices, but the technique was too complicated to warrant its use commonly. Therefore, he selected a stock vaccine composed of the common pathological organism or of a mixture when more than one organism might be suspected; he administered that vaccine controlled only by clinical findings. The downfall of vaccine therapy, as suggested in the introduction, began with this practice. Mathews saved time but he almost destroyed the practice of using vaccines as therapeutic agents.

In 1907 in a Symposium [6] on Vaccine Therapy by American Physicians, their general consensus of opinion was that vaccine therapy had been too much exploited, and Wright's technique was too involved. Moreover, they felt that the opsonic theory and vaccine therapy were two different matters.

The use of stock vaccines in the succeeding years was as much exploited as previously had been the vaccines of Wright. The preparation of stock vaccines was commercialized, and they reached a status little better than quackery. Even Sherman [7] advocated autogenous vaccines as preferable but found that the time element was excessive, and the cost prohibitive for patients of

ordinary means. There seems to be no remedy for these two important disadvantages. Therefore, the use of autogenous vaccines was necessarily limited to subacute and chronic infections not amenable to stock vaccines or to those cases presenting an unusual organism. The stock vaccines composed of various mixtures of many organisms gave very irregular results. Occasionally a case was benefitted but no consistent favorable prognosis could be formed.

This likewise seems to express our own attitude toward vaccines until the past five years. Wherever and whenever an autogenous vaccine might be obtained, it was to be preferred; but if one could not be obtained, then a stock vaccine was used which, with good fortune, might give the result desired.

According to Ludwig Hektoen [8] in 1929, the modern consensus of opinion of practicing physicians disfavored vaccine therapy of any sort. He gives results on a questionnaire received from 1261 physicians.

"Of the 1261 physicians canvassed, only 17 favored the use of vaccine therapy at all.

"Of the 1261, 430 either did not use or never had used autogenous vaccines for any purpose; 142 had used them rarely and 172 had abandoned the use of autogenous vaccines.

"Of the 1261, 577 either did not use or never had used stock vaccines; 49 had used them rarely; 198 had abandoned them because of failure to obtain desired results.

"In the sixty-three different disease conditions in which the use of stock polyvalent vaccines was reported, the negative or inconclusive results greatly out-numbered the good results.

"Of the 1261 physicians, 140 or eleven per cent reported untoward or harmful effects from the use of stock polyvalent vaccines.

"Seventeen cases of asthma are reported to have followed courses of bacterial vaccines, administered to patients who previously were not known to have suffered from asthma".

Even before the work of Wright, observations were being made of a very different phenomenon which we propose to extend to vaccine therapy. In this new light many of Wright's practices still seem practicable though we interpret his results in an entirely different manner. Accordingly we have come to divide all our work into cases of two types: one type which seems to follow the older ideas more closely in that we are trying to establish an immunity against a definite bacterial invasion; and the other type in which we are treating not so particularly to immunize as to relieve fixed tissues of

sensitivity.

During the last fifteen years this work has been sponsored largely by Besredka of the Pasteur Institute, Paris. As early as 1798 Jenner observed what we term allergy in connection with small-pox vaccination; Magendi in 1839 observed a similar phenomenon as he worked with rabbits and egg-albumen; in 1895 it was again observed by Brieger[9] who worked with tetanus in goats; by von Behring and Kitashima [10] with diphtheria in horses; but to Hericourt and Richet [11], and later Richet and Portier [12], is due the credit for the really fundamental observations upon which we base our present knowledge of anaphylaxis, as well as the term itself, which means "without protection".

Hericourt and Richet in 1898 found that repeated injections of eel serum into dogs gave rise to increased susceptibility rather than immunity. These studies were continued in 1902 by Richet with Portier whose experiments on actino-congestin have become classic. By injecting first a non-toxic dose of actino-congestin, which caused no symptoms whatsoever in the dog, and then some twenty-two days later injecting a second dose even one-eightieth as large as the first, there resulted immediate symptoms and death in thirty-five minutes.

Arthus in 1903 [13] published an account of his observations in which he noted that non-toxic substances

also produced symptoms like those previously observed by Richet. He found that anaphylaxis was specific; milk and serum were not interchangeable in the process.

In 1903 von Pirquet and Schick [14] made rather poignant clinical observations which related to hypersensitiveness of the human subject to antitoxin for diphtheria. This never appeared before six days and usually from the seventh to the twelfth day. It was von Pirquet who proposed the term allergy meaning "altered energy".

Theobald Smith [15a] observed a remarkable sensitiveness to horse serum in guinea-pigs used for the testing of anti-diphtheritic serum. Otto in Germany [15b] and Rosenau and Anderson in America studied this phenomenon in some detail. Otto noted that the specificity of the sensitivity was toward horse serum alone and not to the serum of any other animal: like Arthus, he was finding that sensitivity is specific. Rosenau and Anderson succeeded in rendering guinea-pigs immume to anaphylactic symptoms by injecting them with massive doses of the given serum during the incubation period which lasted 10 days. If so treated, the result seemed to be a prolongation of the incubation period. If untreated, the hypersensitive state of the guinea-pig lasted for months. Like Otto, they,too,found the reaction to be specific.

During the years 1907 to 1912 we find the story of anaphylaxis continued by Besredka and Steinhardt who accomplished active sensitization by a single injection of serum given either subcutaneously or intraperitoneally. Furthermore, to sensitize rapidly and satisfactorily weak doses were given. It was thought that dilution in the body might play an important role in sensitization, greater dilution inducing greater sensitization but there was soon established a limit above which further dilution gave uncertain results. Besredka and Steinhardt found that diluted serum was more resistant to coagulation by high temperature than undiluted serum, and the sensitizing power was unaffected by heating to 100° C. or even 120° C. which exceeds the temperature of coagulation by far.

Passive anaphylaxis may be conferred upon an animal by injecting into it the serum of another animal which has been actively sensitized. It is similar to active anaphylaxis except in one respect, it is produced almost immediately, the incubation period being very short.

Besredka [18] noted that in the case of the same serum, the intensity of the reaction is in direct ratio to the quantity injected. This suggested to him the idea of graduating the dosage of therapeutic and normal sera. Each serum had its own degree of toxicity which could easily be determined. Toxicity of a serum is partly due to the age of the serum, but most particularly to the

individual nature of the serum. That toxicity due to age begins to lessen at ten days and by six weeks to two months, ceases to be a factor. Heating diminishes the the toxicity of sera. Preservatives as used, particularly 0.5 per cent phenol, do not affect the toxicity of serum.

Thus Besredka was presented with two methods of attack to the problem of preservation from anaphylactic shock; attenuation of the toxicity of sera for injection, or the production of an immunity in the animal against sera. Rosenau, Anderson, Otto and Steinhardt each in turn tried to vaccinate the guinea-pig against the toxin by giving a series of massive injections. The guinea-pig was found not sensitive. But Besredka found that a single injection conferred protection against anaphylaxis, thereby refuting the toxin phase of the controversy. After a very small dose the animal can, within a few minutes, tolerate a much larger dose which in turn serves to protect the animal against a still larger dose. In this manner the amount of serum injected, taken cumulatively, becomes a very large amount compared to the original lethal dose. This is called the method of graduated small doses. Passively sensitized guinea-pigs could be desensitized, too, by an intraperitoneal injection followed by an intravenous injection, or subcutaneously.

This method has been applied to protect an animal from death by anaphylaxis before an anti-bacterial immune serum could be attained.

The analogy between serum anaphylaxis and bacterial anaphylaxis has been studied by Zinsser and Parker [19] who were of the opinion that the two are analogous. This analogy involves comparing the two in respect to the production of active anaphylaxis; the production of passive anaphylaxis; and specificity. They point out the work of Kraus and Doerr[20] who were able to actively sensitize guinea-pigs to typhoid cultures, paratyphoid cultures, and cholera toxin. Delanoë [21] also sensitized guinea-pigs to typhoid cultures, paratyphoid cultures, and the colon bacillus. Holobuth [22] sensitized guinea-pigs using repeated small doses of bacteria injected subcutaneously for ten days. The test injection was given intravenously three weeks after the last subcutaneous injection. A large dose is required as a test injection to bring about the usual characteristic symptoms of anaphylaxis which may even prove fatal.

They also refer to the work of Rosenau and Anderson who used extracts of Bacillus coli, of yeasts, of Bacillus subtilis, and of the typhoid bacillus injecting these subcutaneously; then nineteen to thirty-five days later they reinjected intraperitoneally or subcutaneously and obtained mild, marked, or severe reactions but rarely death.

Kraus and his fellow workers performed the first experiments on passive bacterial anaphylaxis. They succeeded in conferring passive anaphylaxis upon guinea-pigs but injecting into them the serum of a rabbit actively sensitized by injections of increasing doses of typhoid bacilli fifteen days previous. Various other experimenters produced passive anaphylaxis using other organisms such as the meningococcus, the plague bacillus, and the cholera vibrio.

Kraus and Doerr held the reaction to be rigidly specific; Delanoë [21] observed more marked reactions with the homologous organism but found some group sensitivity. Kraus and Amiradzibi [23] in 1910 found the specificity to be rigid enough to differentiate between particular strains of an organism. Besredka also believes that the specificity is exceedingly rigid.

Zinsser and Parker found bacterial anaphylaxis strictly analogous to serum anaphylaxis but noted in their experiments that whenever the antigen was a cellular one, as in the case of bacteria, there are two phases to the phenomenon of hypersusceptibility: First, the cell constituents must be liberated before they can act as antigen; then the process may be similar to serum anaphylaxis, except that the sensitization is accomplished by repeated injections of antigen just as might occur in infectious diseases where there is a continuous liberation

of fluctuating amounts of specific antigen in a body at first unsensitized, but later increasingly hypersusceptible and perhaps repeatedly sensitized and desensitized during the course of the disease.

Thus, the only discrepancy between serum anaphylaxis and bacterial anaphylaxis is in the toxicity of the first inoculation; the serum is non-toxic, but the first dose of bacteria may even cause death.

Zinsser and Parker summarized the results of their experiments in this manner:

1. Sensitization of the tissues of a guinea-pig, as noted in the isolated uterus, is possible and requires from three to five days even when passive sensitization is used.

2. The sensitized uterus reacts not at all with whole bacteria or whole red cells showing that the solution of bacteria must be brought about before there is a reaction with sensitized organs.

3. The sickness brought on by the initial dose of typhoid antigen in a normal animal is probably not due to tissue sensitiveness but perhaps to an intravascular reaction.

Besredka came to regard anaphylaxis as a cellular phenomenon, the entire reaction being intracellular. He is inclined to explain it hazily as a

matter of colloid chemistry related or unrelated to present ideas of immunity.

A similar idea is carried further by W. B. Wherry [24] who applies it in his theory of "Tissue Hydration and its Relation to Hypersusceptibility and Immunity". Like Martin H. Fischer,[25] he regards the tissues as emulsion colloids and feels that the invading organism must be capable of freeing water in the tissues in order to grow there. Such water of syneresis as is liberated contains food in solution and in such a state only is it suitable for use by bacteria. It is well known that organisms frequently grow in the water of syneresis, condensation water. Likewise those organisms which can produce an oedema, can invade tissues more rapidly. There is considerable controversy as to the identity of the substance which is responsible for the oedema. This may be histamine which is known to produce just such reactions in dogs and man. Wherry is inclined to believe that the amine is produced within the tissue by the action of the body ferments upon the bacterial bodies, and not by an action of the bacteria, themselves. There is developed within the tissues a toleration for increased quantities and concurrently, an improvement of the infectious process is brought about, according to Wherry, by a decreased tendency to tissue hydration and by phagocytosis.

The tissue hydration theory of infection gave rise to the idea that the skin of susceptible individuals should show local urticaria when injected with dead bacteria or their toxins while immune individuals should not; previous infection would be shown by more marked local reactions to bacteria or their toxins.

The intradermal test had long been used. It seems to have been sponsored by Ch. Mantoux as a method of determining sensitivity to tuberculin about 1908. Subsequently the intradermal test has been directed to determine susceptibility to the toxins of diphtheria and scarlet fever. It is now commonly employed in the medical practice for that purpose but no one seems to have emphasized the fact that this same test may be used to indicate susceptibility to other bacterial organisms.

Wherry [26] in one of his papers reports a number of cases on whom he worked with this in mind. Among them were eight cases of asthma, six cases of sinusitis, six of mucous colitis, two of urticaria, one of blepharitis, and one of angio-neurotic oedema. They were all treated with vaccines to which they were found skin sensitive and in general, the results were encouraging. By means of these patients he showed that individuals vary in their reactions to the same organism; and the reactions were specific to the strain.

In the Spring of 1926 the Kuhn Clinical Laboratory of the Cincinnati General Hospital was opened and at the suggestion of W. B. Wherry we started out to supplement his work on intradermal reactions to bacterial vaccines. There were available to us the patients in the hospital, those in the Out-Patient Dispensary as well as the private patients of Doctor Roger Morris and others of this community.

Within a very short time there was so great a demand for treatment with bacterial vaccines that we abandoned the arbitrary testing with stock strains of bacterial vaccines and started to prepare autogenous vaccines, skin testing with the strains isolated, and treating with the strains to which the individual was sensitive. The stock strains were resorted to only when we failed to secure satisfactory results with the autogenous strains. Very often the patients were referred to us after the primary focus had been removed, when improvement had not been obtained by that procedure. The vaccines unclaimed and those not used served as stock strains. For testing with stock strains usually various strains of the same organism were mixed to obtain a "type polyvalent vaccine". Of course, autogenous strains were preferred, but occasionally it is necessary to resort to stock strains as the organism

may not still be an active invader though the patient is still sensitive.

Not all cases submitted are worthy of vaccine therapy, but from those submitted, interesting groups may be worked out.

Wherry had been using an immediate dermal reaction rather than the delayed or twenty-four hour reaction as an index. This immediate reaction, we came to regard as indicating merely susceptibility to an organism and as such it did not concern us; delayed reaction, we believe, indicates a definite invasion and inadequate protection or sensitivity. It is with this reaction that we are concerned. This is similar to the reactions observed in the tuberculin reaction.

There has been no attempt to correlate our treatment with phagocytosis, but in those cases treated with autogenous vaccines to which they are not skin sensitive, the improvement must be regarded as due to the production of immunity rather than a desensitization.

In general our method has been to desensitize symptomatically and then continue in an attempt to render these patients completely desensitized, indicated by a negative skin reaction.

II. Experimental.

Specimens. Fresh specimens in sterile containers

were required to be sent to the laboratory as soon as possible.

Cultivation. For cultivation inoculations were made into dextrose ascites broth; upon dextrose ascites agar, aerobically; upon dextrose ascites agar at partial tension with Bacillus subtilis; upon a dextrose ascites agar or a blood plate. In addition stools were inoculated into the brilliant green bile medium of Muer and Harris [27], and onto an Endo's plate, the Stitt [28] modification. Anaerobic cultures with the method of Rockwell [29] were used when indicated. Enriched dehydrated media has been used throughout. The nutrient broth and agar are enriched by one per cent of glucose and 7.5 to 15 per cent of ascites, depending upon the quantity required to accelerate growth.

Ascites for this purpose is obtained from hospital patients. The specific gravity is brought to 1.010. Then 10 c.c. of normal sodium hydroxide per liter is added and the mixture is heated for an hour in an Arnold Sterilizer, then filtered to remove any coagulum. It is flasked in amounts convenient for usage, and autoclaved for twenty minutes at a pressure below fifteen pounds. Some ascites gels in the Arnold and must be discarded.

Ascites so prepared retains its power to enhance growth for many months. Media enriched with

dextrose and ascites as described will grow the meningococcus and the gonococcus. It may be substituted for blood agar except when dealing with the pneumococci and the influenza bacillus.

This dextrose ascites broth favors the streptococcus while inhibiting the staphylococcus and the gram-negative rods. Streptococci which grow as sub-surface colonies on agar may be obtained from such broth culture.

Agar plates serve as a quick means of isolation; the blood agar plate grows special organisms and shows hemolysis; pigment formation has been observed upon Loeffler's blood serum; the Endo plate may be used before or after isolation in the brilliant-green-bile medium, serving to differentiate between the gram-negative rods.

Partial tension cultures grown in tandem with the Bacillus subtilis give altered concentration of oxygen and carbon dioxide, which vary as the subtilis grows, and at some stage promote the growth of specific organisms. The two slants are attached by means of a piece of large rubber tubing. Successive transplants may usually be made aerobically.

When the anaerobic tube method is used, great care must be taken in removing the growth with a platinum loop not to remove any of the chemical as well.

Preparation of Vaccines.

Whenever possible a twenty-four hour growth is used. For organisms which grow well, 10 c.c. of sterile saline is added per slant. For others, the amount is decreased so that the suspensions are comparable.

Suspensions are made in sterile physiological saline, 0.85 per cent sodium chloride.

The broth cultures may be used if the ascites, which precipitates out as growth takes place, is removed by centrifuging at a low speed for a short time. The organisms remain suspended and they are then centrifuged out and washed at least three times with sterile saline before the final saline suspension is made.

Suspensions are shaken to break up gross clumps and then filtered thru sterile cotton. This is accomplished by slowly shoving a small plug of sterile cotton to the bottom of the tube with a sterile pipette, and drawing the suspension through the cotton into the pipette. Some streptococci must be carefully suspended, washed, and used without filtering as they are entirely retained by the cotton.

Sterilization of the vaccines is carried out by heating to 60° - 65° C. for one hour on successive days until the controls are negative. Controls must be made into an enriched medium and at the optimum tension for

the particular organism. Not less than 0.2 c.c. has been used as a control.

When sterile they are preserved with 0.5 per cent phenol. The rubber caps are sterilized by boiling in 5 per cent phenol and subsequent standing for twenty-four hours in that phenol. After capping, the vaccine must again be controlled for sterility.

Intradermal Testing.

The intradermal test consists of introducing one minim of the vaccine to be tested into the dermis of the inner side of the fore-arm, taking great care to avoid veins and capillaries. This is done with a small hypodermic syringe of the tuberculin type to insure careful measurement of very small quantities. The ordinary hypodermic syringe is not sufficiently accurate. The needle used is a regular fine hypodermic needle gauge 24 or finer. A small white elevated area results if the injection has been correctly made. Within a few minutes a small area of oedema and a surrounding area of erythema appear. All injections bring about a small area of erythema but with negative reactions, these soon fade; with positive reactions these areas increase in size, with swelling, local heat, intense redness and persist even after twenty-four hours. After forty-eight hours

reactions usually subside but occasionally one may increase in size even after forty-eight hours.

In some instances the injections into the skin are followed by symptoms of a general type, rise in temperature, malaise, headache and nausea or frequently specific attacks.

Reading the Reactions.

In reading the reactions we refer to them as negative, which shows only evidence of the injection of the needle at twenty-four hours; mild, characterized by an area of redness no more than one cm. in diameter; moderate, having an area of from 1 cm. to 3 cm. in diameter; marked, extending to half way around the arm; severe, half way around the arm and up the arm to the axillary lymph glands. At times there is also a general reaction and the patient may develop a rise in temperature, headache, malaise and nausea, or a focal reaction reproducing the symptoms for which he is about to be treated. Such reactions are unfortunate since the patient who most needs treatment becomes frightened and sometimes refuses to cooperate.

Mixing the Vaccines.

The vaccines to which the patient is sensitive are mixed in an inverse ratio to the degree of sensitivity.

A test injection of one minim of the mixed vaccine is made. The vaccine is then diluted until the local reaction is slight and there are no general symptoms. It is again controlled for sterility.

Treatment

The initial dose of the mixed vaccine is one minim given intradermally or subcutaneously. Often times further dilution is advisable. Occasionally we start with 0.5 minim doses rather than dilute the vaccine further. Again a small amount of very dilute vaccine may be prepared to start the treatment, subsequently changing to the more concentrated mixture. There is less danger of ill effects from a slight variation in dosage if the vaccine is very dilute.

When the sensitivity is marked, daily injections are better, continued until the reaction definitely decreases; otherwise injections on alternate days is satisfactory. Increases of 0.5 minim or one minim according to the sensitivity are made no oftener than weekly and sometimes increases are not indicated for several weeks. An additional 0.5 minim may bring on a critical attack in these exceedingly sensitized individuals.

It is most important to avoid severe reactions of either the local or general type since we have shown repeatedly that such reactions seem to step up the

sensitivity and certainly operated against the process of desensitization.

Treatment is continued until there is freedom from the original symptoms; further treatment until the patient loses entirely the skin sensitivity is desirable but often before that time the patient has been symptomatically relieved, and sees no benefit to be derived from further treatment.

III. Application

The experimental application of our theories has been studied in various groups of diseases of which we are presenting six: Bronchial asthma, sinusitis, arthritis, angioneurotic oedema, non-ulcerative colitis, and certain skin infections. Of these, bronchial asthma is presented first, and in greatest detail since, from the very nature of the symptoms, it offers the best opportunity for observing definite progress.

A. Bronchial Asthma

Bronchial asthma was brought into the category of anaphylactic phenomena in 1909 by Auer and Lewis [30] who produced in a guinea-pig an attack typical of asthma in humans. This was accomplished by a second injection of horse-serum into a guinea-pig

previously sensitized to that serum. The guinea-pig displays symptoms similar to those in man. The respiratory dynamics may be entirely explained as the total result of an acute bronchiolar hypertonus. Due to the spasm in the terminal bronchiolar tree, the alveolar air is not displaced, the lungs are held in a semi-inspiratory position and the mechanical disadvantage is maintained, primarily, in the expiratory phase. The underlying spasm has been demonstrated, beyond question, to be the reaction of a sensitized bronchiolar tree to a specific sensitizing substance.

Asthmatics may be sensitive to various substances, usually protein in nature, including pollens which are inhaled, foods eaten, or bacterial proteins resulting from auto-infections. These subjects give skin reactions to the sensitizing antigen. These reactions are used by physicians generally to determine the substance, more commonly a food, or pollen, to which the individual is sensitive. Recognition of bacterial sensitization is evidenced by the fact that stock bacterial antigens are included with the pollens and foods but these are seldom used for desensitization. More rarely are autogenous bacterial antigens employed even for test purposes.

Walker [31] suggested that the incidence of bacterial asthma was rather low, only 8 per cent. Lambright [32] in a recent discussion concluded that

bacterial asthma is not common; the cutaneous reaction, in his opinion, does not prove the etiology of the substance used; "vaccines should be used in conjunction with other means to relieve the condition; the bacterial treatment of asthma cannot be compared with the relief obtained by desensitization to pollens". Contrarily, we are certain that bacterial asthma is much more common than is ordinarily supposed; the cutaneous reaction to bacterial antigens has been our only means of selection and the causative relationship of the antigen is particularly confirmed by provoking a typical asthmatic attack in the susceptible individual by either the intradermal test or by injections used in treatment. A definite relation is indicated between the cutaneous reaction and the disease itself; our patients have received no other treatment except for ephedrine to check an accidental attack; and from the very first they are usually relieved.

Bacterial asthma very frequently has its onset following such acute infections as whooping cough or influenza. It may occur among young children, particularly among those with an hereditary tendency toward sensitization, but it is more prevalent among adults having no asthmatic history previous to an acute infection. Repeated colds seem to favor the development of asthma. The subsequent attacks are preceded by a second infection

analogous to a second injection of serum in the animal experiments. As a preliminary step, sensitivity to the pollens and foods must always be first excluded. The sensitizing organisms vary and usually sensitization demonstrated is to but one or a very few organisms, in a given case. Vaccines are prepared from material obtained from the primary focus whenever possible. If the primary focus has been surgically removed and the patient still continues to have attacks of asthma, as is very frequently the case, material from the bronchi offers the most likely source from which to recover the offending organism. Repeatedly in our series, the ethmoid sinuses have harbored the sensitizing strains and material from the post-nasal drip, or obtained by post-nasal tampoon, has yielded them, often in pure culture.

Kolmer [33] recognizes the existence of bacterial asthma and suggests a method of treatment similar to the one employed by us. However, it has been our experience that extremely sensitive individuals could never be handled in the manner described by him. Successful treatment of sensitive cases is only accomplished by frequent injections of extremely small doses of a very dilute suspension of the organisms to which the patient is sensitive. No more than daily injections are practicable for our patients and usually injections on every second day sufficed. Months of daily treatment may

intervene before the interval between injections can be lengthened. If the interval be prolonged, inadvertently, the patient often complains of shortness of breath. Seldom have we been able to give injections less than three times weekly.

When autogenous vaccines have failed to demonstrate the particular organism or organisms to which the patient was sensitive, we have had recourse to a large series of stock bacterial vaccines consisting of those organisms most often found in the upper respiratory infections:

1. Staphylococcus aureus
2. Streptococcus hemolyticus
3. Pneumococcus, type I
4. Pneumococcus, type II
5. Pneumococcus, type III
6. Pneumococcus, type IV
7. Micrococcus flavus
8. Micrococcus catarrhalis
9. Micrococcus pharyngis siccus
10. Bacillus influenzae
11. Friedländer's bacillus
12. Bacillus duplex non-liquefaciens
13. Bacillus abortus (Bang).

Of these only the ones which caused a definite reaction were used in the preparation of the patient's vaccine.

Of all our cases, the asthmatics are the most difficult to handle. Attacks of asthma may be spontaneous in the early part of the course of treatment, or may be due to a slight overdosage of vaccine. In either case, ephedrine or epinephrine has been employed to control the attack. If due to overdosage, and also after a

spontaneous attack, the amount must be cut down to a minimum before re-attempting desensitization.

Coca [34] refers to the lessened clinical sensitiveness as "hyposensitization". He does not recognize it as desensitization for he claims there is no disappearance of the sensitizing substance. He fails to consider that the patient may re-sensitize himself to the same organism even during the treatment unless the focus is removed. It is obvious that whenever possible, the focus should be removed in order to prevent further sensitization.

To substantiate our claims we present a series of eleven cases of bronchial asthma, successfully treated by the method given above; a twelfth case is under observation at present. These patients have all been tested to the pollens and foods. Each gives a history of asthma following an acute infection or repeated colds. They are all cases of long standing with an average duration of 7.5 years, the shortest being nine months and the longest fifteen years. We have followed most of them for 1 to 2.5 years and no case is included which has been followed less than eight months.

Table I.

Case	Duration	Treatment	Report.
1	5 yrs.	2½ yrs.	No recurrence.
2	9 yrs.	2½ yrs.	No recurrence.
3	1 yr.	2 yrs.	No recurrence.
4	9 mo's.	9 mo's.	No recurrence reported.
5	6 yrs.	9 mo's.	Controlled- no attacks.
6	6 yrs.	8 mo's.	Controlled- marked improvement.
7	6 yrs.	8 mo's.	Controlled- improvement very marked.
8	5 yrs.	1 year.	No attack for last 7 months.
9	15 yrs.	1 year.	No attack for last 6 months.
10	10 yrs.	1½ year	No recurrence.
11	3 yrs.	1 year.	Improved.
12	1 year.	3 mo's.	Slight improvement.

Table II

Autogenous Antigens	CASE 1	2	3	4	5	6	7	8	9	10	11	12
Staphylococcus aureus	++	+			++	++						++++
Staphylococcus albus		+	+								+++	
Staphylococcus, hemolytic							+	+++	++++			
Streptococcus hemolyticus	+++		+	+					++	++		
Pneumococcus, type 1	+							++				
Pneumococcus, type 111	+											
Micrococcus tetragenous												++++
Gram positive diplococcus					++++							++++
Micrococcus catarrhalis										+++		
Bacillus of Friedländer					++++	++++	++++			++		++++
Bacillus influenzae							+++					
Bacillus coli communior	+++											
Bacillus coli communis	+++											
Gram positive rod - not classified			+									

Table III

Stock Antigens	CASE 1	2	3	4	5	6	7	8	9	10	11	12
Staphylococcus aureus	++			++								
Streptococcus hemolyticus	+		++	++								
Pneumococcus, type I	+++											
Pneumococcus, type II												
Pneumococcus, type III	++											
Pneumococcus, type IV												
Micrococcus flavus	++	++										
Micrococcus catarrhalis	+++											
Micrococcus pharyngis siccus				+++						+++		
Bacillus influenzae			++									
Bacillus of Friedländer	++++	++	++	++++						+++		
Bacillus duplex non-liquefaciens		++		++								
Bacillus abortus (Bang)	++++			++						+++		

Case 1. A high school girl, 17 years old. Asthmatic attacks occurring at monthly intervals followed whooping cough at 12 years; these attacks were often preceded by a cold and sore throat, and during the attacks, hospitalization was necessary for several days. Thruout the intermissions she was quite well except for a low grade chronic bronchitis which persisted.

Cultures were made from her tonsils, removed in November, 1927, nose, throat, sputum and stool. Vaccines were prepared from the organisms yielded by these specimens.

She was found to be moderately sensitive to Staphylococcus aureus, Streptococcus hemolyticus, Bacillus coli communior, Bacillus coli communis; and slightly sensitive to Pneumococcus, type I and type II.

Treatment was started with a mixture of these vaccines, to which the patient was quite sensitive. A flare up of the original dermal reactions occurred at the end of ten days. The first regular monthly attack was less severe and did not necessitate hospitalization. Six weeks elapsed before the second attack which was still less severe. The injections were made daily, the amount being controlled by the local reaction. No severe attacks were ever brought on by an overdosage of vaccine, but if the interval were prolonged, the patient complained of

shortness of breath. She became gradually less sensitive to the mixture and was receiving four minims at the end of four months.

At this stage we were rather disappointed because we had not been able to completely desensitize the patient, as shown by her shortness of breath when the interval was prolonged. Of course, she was somewhat improved in that her attacks were less frequent and less severe, but in general the process of desensitization was consuming more time than we had anticipated. For this reason we thought best to test her sensitivity to our stock bacterial antigens to see if we had not perhaps missed an important organism. Her sensitivity to Staphylococcus aureus was about the same, but she was less sensitive to the Streptococcus hemolyticus, even more sensitive to the Pneumococcus, types I and III, of our stock strains than to her own. She was moderately sensitive to Micrococcus flavus, and Micrococcus catarrhalis, markedly sensitive to the Bacillus of Friedländer, and Bacillus abortus. These vaccines were added to her own autogenous mixture; treatment was resumed and continued until June. During this time injections were made three times weekly; no less would keep her free of dyspnea.

During the summer of 1928, the patient was working and therefore unable to come to us for treatment.

The house physician who had attended her in the hospital gave her the vaccine outside the hospital but he reported that her visits were very irregular. Consequently she experienced two unusually severe attacks in the early Fall.

She then returned to us for daily injections of very small doses. The same process was repeated but the time required was shorter.

Although treatment was given regularly thruout the year until June, 1929, no more than 4.5 minims was ever tolerated. She was still somewhat skin sensitive, but her general condition was tremendously improved, she had had no further attacks of asthma during that year, and she soon failed to return for more treatment. We rather expected her to have a recurrence since she was still somewhat skin sensitive to the vaccine, but so far as we know she has been entirely free from asthma.

This was as obstinate a case to handle we have encountered. The twelfth case, now under observation, presumes to be equally as hard to handle.

In summarizing this case it might be termed a severe case of asthma of long standing. It was particularly difficult to control, probably rendered more difficult by enforced periods of non-treatment. The patient was symptomatically cured, though still somewhat skin sensitive.

Case 2. A medical student, 22 years old, whose father was a physician and had usually prescribed for him. After influenza in 1918, the boy had had chronic bronchitis with occasional attacks of asthma occurring at any season of the year. These attacks were preceded by a cold, but were usually of short duration. Former attacks had been easily controlled with adrenalin or ephedrine, but in this instance the attack was not so controlled and he was brought into the hospital in November, 1927.

The dermal tests for pollens and foods had been run previously without showing sensitivity.

Cultures were made from sputum. The patient gave no reaction to Streptococcus hemolyticus or Micrococcus pharyngis siccus and only slight reactions to Staphylococcus albus and Staphylococcus aureus.

Since he showed so little sensitivity to his autogenous strains, he was tried with the stock vaccines. Of these, he was sensitive to the Micrococcus flavus, the Micrococcus catarrhalis, the Bacillus of Friedländer, and Bacillus duplex non-liquefaciens.

Treatment consisted of daily injections of a mixture of these strains for two months. At first there was a moderate local reaction but never any specific attack. He was soon able to take 8 minims with no difficulty. Treatment terminated in April, 1928.

For the remainder of that year and thruout the school year, 1928-1929, the patient had no further attacks.

This was a mild case of asthma of long duration. The patient was but slightly sensitive to his autogenous strains, so that stock vaccines selected by their skin reactions were used in treatment. The case was easily and satisfactorily controlled in a short time. This could hardly be considered a remission since it has lasted for over two years, when one considers the previous history. There was evidently a familial tendency toward sensitization as a younger brother also developed asthma about the time we were treating this case.

Case 3. A man, 41 years old, having bronchial asthma with chronic sinusitis and tonsillitis. One month after an acute attack of bronchitis in January, 1927, he developed asthma. A few weeks intervened between attacks; during that time he was fairly well and free from symptoms of asthma.

A severe attack in March, 1928, necessitated hospitalization. At that time he was tested with the pollens and various foods but no sensitivity was indicated. Several teeth were extracted, and a radical antrum operation was performed before he was referred to

the laboratory. It was necessary to use sputum cultures as a source of material. He was but very slightly sensitive to a Staphylococcus albus, an hemolytic streptococcus, and an unidentified Gram positive rod isolated from this source.

Treatment was started in April, 1928. At first very small doses of 1 minim or less, brought on asthmatic attacks of a very severe type. Dilution of the vaccine was indicated until he could tolerate 1 minim of the diluted vaccine without a specific attack and only slight local reaction. In May, 1928, a slight overdosage of vaccine, we suspect, caused the patient to fear an attack of asthma. He came into the hospital but was soon discharged, since the attack failed to materialize.

In July he was retested to our stock vaccines and we found that his sensitivity to the Streptococcus hemolyticus had disappeared; but he had retained his sensitivity to the Bacillus of Friedländer, and Bacillus influenzae.

Treatment was continued until October 1, 1928, when the patient felt able to return to work. No further recurrences have been reported.

This case, though of shorter duration, was a severe one. After the focus had been removed, the patient retained an extreme sensitivity which was easily controlled, undoubtedly due to the fact that the focus

had been removed and he therefore, was not being resensitized. He was able to return to work sooner than anticipated, but deemed it advisable to continue treatment until we felt sure there would be no recurrence.

Case 4. A colored woman, 37 years old, with chronic bronchitis and asthma following a severe cold. Shortness of breath was always associated with a fresh cold, and occurred irregularly. The patient had been unable to work for the nine months previous, for she had developed a status asthmaticus. She was referred to the laboratory in October, 1927.

She was sensitive to an hemolytic streptococcus recovered in pure culture from sputum.

This vaccine was given to her until March, 1928, three times weekly. She tolerated any amount up to 7 minims very well but at that point slight attacks might be brought on by repeated injections. She was sufficiently improved to resume work for part time.

When tested for sensitivity to our stock vaccines in April, 1928, she was still sensitive to the hemolytic streptococcus; moderately sensitive to Staphylococcus aureus, Bacillus duplex non-liquefaciens, and Bacillus abortus; and markedly sensitive to the Bacillus of Friedländer, and Micrococcus pharyngis siccus.

These were added to her autogenous vaccine; treatment was resumed and continued until January, 1929. Patient was much improved generally and able to work.

This was a case of bacterial asthma, easily and quickly controlled. Improvement was rapid and lasting.

Case 5. A woman, 30 years old. She was referred to the laboratory as an asthmatic with chronic sinusitis. During July, 1929, she was hospitalized for an antrum wash which yielded an hemolytic Bacillus of Friedländer to which she was markedly sensitive.

Treatment was begun in July, 1929, and is being continued three times weekly.

Nose and throat cultures taken in March, 1930, for the purpose of preparing more vaccine again yielded the Friedländer bacillus, with a Staphylococcus aureus and a Gram positive diplococcus. Her reaction to the Friedländer bacillus had diminished but was still marked. Sensitivity to the Staphylococcus aureus was moderate, and to the Gram positive diplococcus it was marked. The patient is now receiving a mixture of these organisms.

Even though she has had several colds this winter, there has been no recurrence of her asthmatic attacks for the period of nine months.

This was a definite asthmatic who displayed skin sensitivity to one organism particularly and that from an antrum. Vaccine was well tolerated and improvement, marked from the beginning, was probably due to a combination of the removal of the focus and a desensitization process as well. Sensitization was undoubtedly being brought about by the Friedländer's baccillus found in pure culture in the right antrum.

Case 6. A woman, 56 years old, with chronic asthma dating back to an infection, possibly adult whooping cough, in 1924. This patient had had asthma previously extending over a period of about ten years. The removal of tonsils, appendix, and gall bladder had evidently aided in clearing up the former asthma. In August, 1927, she reported to the Clinic that her attacks had recurred and were becoming more frequent and severe. The case was referred to us in August, 1929.

This last asthmatic period seems to have been brought on by sensitization to a Friedländer's bacillus, and a Staphylococcus aureus recovered from an antrum wash. She was sensitive to both of them.

During the first few months of treatment we were unable to correlate her attacks of asthma with the injections since she was in a continual asthmatic state. Gradually, however, her asthma became less severe, and

at the same time, she seemed to improve generally. Recently her asthma seemed to be related to recurring colds but manifests itself as a slight shortness of breath which does not prevent her coming for treatments.

In April, 1930, she was again tested to her original strains isolated, and found moderately sensitive to Staphylococcus aureus and markedly sensitive to the Friedländer's bacillus.

The marked improvement in this patient warrants further treatment which is advised since she is still very sensitive to her own strains.

Case 7. A wealthy farmer, aged 57. There is no history of cardio-respiratory symptoms, other than an occasional cold, prior to the onset of his present illness. In 1924, four years before we first saw him, he had an attack of illness diagnosed as influenza. Before he had fully recovered from this, asthmatic symptoms appeared ushered in with "wheezing and shortness of breath". Within a year he was having definite paroxysmal attacks of asthma which were coming on with increasing frequency. This was followed by three years of travel undertaken in an unsuccessful attempt to find a beneficial climate.

There was no history of seasonal periodicity, no history of reaction to certain foods but he was, nevertheless, tested to the usual food proteins and

pollens. All these tests were negative and, since his speciality was fine horses, he was carefully tested with extracts made from the hair of each of his horses without showing any reaction.

Having exhausted this field, we cultured the naso-pharynx and sputum obtained after a paroxysm of coughing. He gave positive reactions to the following organisms recovered from material so obtained: Bacillus influenzae, a marked reaction; the Bacillus of Friedländer, a marked reaction; and to a hemolytic staphylococcus, a slight reaction. He has been under treatment for 8 months with marked improvement.

When first seen he presented a picture closely bordering on the status asthmaticus. Under treatment the attacks have become less and less frequent and more mild in type. He has not had an attack calling for the injection of adrenalin in 6 months, and is now driving his horses from thirty to forty miles a day, a thing he had not been able to do for over three years. He is no longer sensitive to Bacillus influenzae, nor the hemolytic staphylococcus, and is less sensitive to the Bacillus of Friedländer than when first tested.

Case 8. A girl of 8 years. The onset of her asthma followed whooping cough when she was 3 years old. A brother, two years older, also had whooping cough and subsequently developed asthma. In this latter instance, however, the condition disappeared spontaneously. There was again no seasonal variation; the patient had previously been subjected to the usual routine tests and had been advised to stop eating nuts. Obedience to this suggestion did not result in any improvement in her condition.

Cultures were made from post-nasal secretions, bronchiolar exudate, and from the throat. Skin tests were made with all the organisms recovered and she was found sensitive to a hemolytic streptococcus and markedly sensitive to a hemolytic staphylococcus.

Desensitization, using these strains, was followed by gradual but definite improvement. Within a month she was able to resume her school work, and went from December to May, 1930, without an attack. She then had a severe sore throat followed in a few weeks with a mild attack of asthma. A green producing streptococcus, not previously recovered, was added to her vaccine after obtaining a positive skin test.

Case 9. A white woman of 38, with a history of chronic asthma extending over fifteen years. At frequent intervals throughout this period, she had been incapaci-

tated for work because of the severity of her attacks. By occupation she was a hair-dresser and several years before we began treating her, she had been sensitized to orris root as demonstrated by intradermal reactions and subsequent desensitization had resulted in definite improvement. For eighteen months prior to her first visit her condition had been exceedingly unsatisfactory with frequent severe attacks.

After ruling out food and pollen sensitization, she was subjected to the same type of investigation described in the previous cases. In addition, her tonsils were removed and the antra of Highmore washed out. From the tonsils there was isolated an anaerobic streptococcus to which she was sensitized and from the antra washings we recovered an hemolytic staphylococcus to which she gave a violent reaction. This same staphylococcus was isolated from the sputum.

Desensitization had to be carried out with extremely dilute antigens and even with care, we precipitated several acute attacks by too rapidly advancing the dosage. We began treatment in April, 1929, and she showed gradual improvement until September. At this time she requested that we permit her family physician to carry on her treatment. He was given careful instructions, which he at once disregarded, and gave the

patient several 0.5 c.c. doses of the vaccine instead of 0.1 c.c. as directed. The result was a violent attack of asthma which brought her into the hospital in serious condition. It was then necessary to carry on very slowly but results were very encouraging; from November, 1929 to May, 1930, she has had no asthma.

Case 10. A white woman and considered from a therapeutic standpoint has been one of the most amazing in our series. The patient had for ten years been subject to very frequent attacks of asthma of a most severe type. For the last half of that period she had been a semi-invalid unable to carry on her household duties. She dates the onset of her illness to an attack of grippe followed by a frontal sinus infection. She came to us exceedingly emaciated, and in every sense a case for hospitalization.

Bacteriological investigation yielded the following strains to which she showed sensitivity: A hemolytic streptococcus, the Bacillus of Friedländer and Micrococcus catarrhalis. She also gave reactions to Bacillus abortus and Micrococcus pharyngis siccus of our stock series. After a month of treatment in the hospital, during which time her attacks gradually faded out, she was discharged and treatment continued by her family physician. The vaccine, after the first

month, was given every second day, and the interval was gradually lengthened to two injections weekly. She was treated, in all, for six months. To the present time, eighteen months since treatment was started, she has had no recurrence. A gain of twenty pounds in weight is reported and she is now able to carry on quite as well as she could before the onset of her asthma.

Case 11. A girl of six years, had been the victim of recurring attacks of acute bronchiolar spasm for three years. The onset of her asthma followed whooping cough and each subsequent attack was ushered in with an acute naso-pharyngitis and fever. She had been sent to Florida and later to the West without the desired therapeutic result. Food tests had been repeatedly made but no definite sensitization was demonstrated.

Cultures were made from the posterior nasopharynx during an acute attack and the predominating organism was a hemolytic staphylococcus. This is the only organism, either autogenous or from our stock series, to which she shows skin sensitivity. A year of treatment has resulted in marked improvement. The asthmatic attacks have entirely subsided but she frequently has severe head colds and her skin sensitivity to the staphylococcus though diminished has by no means disappeared. This child should certainly be

carried along with treatment for another six months; we hope to be able to check the too frequent head-colds.

Case 12. A white woman, 41 years old. She has a severe case of asthma of a year's duration following bronchitis and sneezing attacks. This patient also had had mild asthmatic attacks since her childhood following whooping cough. The recent attacks became more severe after the bronchitis.

Sputum cultures were used as a source of organisms. She was markedly sensitive to all of the organisms isolated: Staphylococcus aureus, Streptococcus hemolyticus, Micrococcus tetragenous, a Gram positive diplococcus, and Friedländer's bacillus. A specific attack of asthma was provoked by the skin tests. A mixed vaccine was used with the result that the patient was feeling much better in about a month. Doses of one minim three times weekly were not well tolerated; she developed another attack of asthma and at this time gave more severe local reactions. The vaccine was diluted and 0.5 minim doses given daily seem to be better tolerated. The patient is showing marked improvement and the prognosis is good.

We may add the case of a middle aged colored woman referred to us in the Clinic as an asthmatic.

Autogenous vaccines were prepared from her sputum but she was not sensitive to any of them. She was not sensitive to any of our stock series either. The organisms of her own strains were mixed and given to her nevertheless. There was absolutely no reaction to this mixed vaccine. She continued to have her night attacks of dyspnoea. We suspected that she was not a bacterial asthmatic and this was borne out by a later diagnosis of mitral stenosis with subacute pulmonary congestion. Treatment of the underlying cardiac condition relieved her air hunger. This case is interesting to us only because of the contrast it presents.

B. Sinusitis.

Sinusitis, though frequently acute in its onset, tends to become chronic, particularly in this locality where we may term it endemic. We will note later how it may act as a forerunner to other diseases such as arthritis. As a chronic infection, it has been attributed to the action of an hemolytic Streptococcus of low virulence. Unless drainage be maintained, there may be an invasion of the blood stream, and even where drainage is inadequate, subsequent sensitization to the offending organisms may take place with secondary symptoms as shown in the asthma group.

We have considered only the cases of chronic sinusitis, frequently showing evidence of sensitization. It has been our experience that the organism involved is more frequently an hemolytic Staplylococcus which might pass unnoticed in the usual routine bacteriological procedures. We have frequently picked it out by our sensitivity tests.

Some cases show only a nasal sensitivity; the sinuses not being involved. This is manifested by chronic sneezing.

Again general sensitization may occur and as in one instance result in attacks of tetany. Such attacks are brought on whenever a fresh infection is acquired.

Some of the chronic cases display focal reactions manifested by sneezing spells after injection of the vaccine. Another case had attacks of acute rhinitis as a result of his first injections. In most of the cases, a typical sinus

headache results from the first treatments.

After a few inoculations, these symptoms of sensitivity pass away and the individual is able to tolerate larger doses of vaccine. Less time is required in treating these cases than for the asthmatics. It is more difficult to note progress, but this is indicated by gradually diminished symptoms, and a resistance to fresh infections. These people will require treatment for several months, but the beneficial effects persist throughout another year at least.

The essential points in regard to this group of cases are summarized in the following table:

Case	Diagnosis	Organisms	Reaction	Treatment	
1	Sinusitis, (antrum)	Staphylococcus albus, non-hemolytic	+++	Severe general reaction, small injections. Treatment inadequate.	Sinus cleared. Colds recurred.
2	Sinusitis	Streptococcus hemolyticus	++++ with glandular involvement	Treated for 2 months.	Improved.
3	Sinusitis-tetany, old chronic case	Staphylococcus albus, hemolytic	++++	Treated for 1 year.	Marked improvement.
4	Sinusitis, Arthritis	Strepto-bacillus, transient: Staphylococcus albus, hemolytic	+ ++	Treated for 1 year.	Relieved while under treatment.
5	Sinusitis, post-nasal drip	Staphylococcus albus, hemolytic	+	Treated for 3 months, irregularly.	Moderate improvement.
6	Sinusitis, post-nasal drip	Staphylococcus albus, non-hemolytic	+	Treated for 3 months. (rhinitis)	Marked improvement.
7	Sinusitis, both antra	Staphylococcus albus, hemolytic Staphylococcus albus, non-hemolytic Gram negative rod, not classified	+++ - +	Treated for 4 months. Sneezing attacks when first treated.	Antra remained clear. No further colds. Condition better than for years.
8	Sinusitis, chronic left antrum	Staphylococcus albus. Streptococcus hemolyticus	++ +	Treated for 2 months. Marked general reaction.	Marked improvement.
9	Sinusitis	Staphylococcus aureus, Staphylococcus albus, Gram positive rod	++, ++ ++	Treated for one month by his physician - vaccine given too rapidly.	Improved - Slight recurrence.
10	Sinusitis, antra Arthritis	Staphylococcus albus, Staphylococcus sureus, Streptococcus hemolyticus Bacillus of Friedländer, Gram positive diplococcus	-, - - ++++, ++++	Treated for 9 months	Improved on treatment. Sensitivity to Friedlander's bacillus decreased; Arthritis improved.
11	Sinusitis, frontal and Ethmoid	Staphylococcus albus, hemolytic	++++	Treated for 2 years	Marked improvement.
12	Sinusitis, Antra of Highmore	Staphylococcus albus, hemolytic: Streptococcus, non-hemolytic: Bacillus of Friedländer	++ - ++++	Treated fro 6 months unsatisfactorily. Polyp removed from antrum. Treatment resumed.	Some improvement.
13	Ethmoiditis	Staphylococcus albus, hemolytic	++++	Treated for 2 years.	Free from symptoms with treatment.
14	Sinusitis, frontal and Ethmoid	Pneumococcus type I, Bacillus of Friedlander; Streptococcus, non-hemolytic; Micrococcus catarrhalis	+, +++ - +++	Treated for 2 years.	Marked improvement.
15	Ethmoiditis	Staphylococcus albus, hemolytic	+++	Treated for 4 months.	Little improvement.
16	Sinusitis Subacute pharyngitis	Staphylococcus aureus, Staphylococcus albus, hemolytic	++ +++	Treated for 3 months.	Improved.
17	Ethmoiditis	Staphylococcus aureus; Staphylococcus albus, hemolytic; Micrococcus pharyngis siccus	++, +++ +++	Treated for 6 months.	Sensitivity decreases. No improvement. Old chronic case?

C. Arthritis.

We have been interested, in this field, in a type of case which has long confused both the internist and the orthopedist. These patients do not have a frank infection with purulent exudate into the joint involved nor are they of that group which shows progressive deforming changes. The outstanding characteristic in this group with which we have been concerned is a sterile effusion into one or many joints. This effusion most frequently occurs in the knee, elbow, or ankle but may involve practically any joint cavity. They have been most successfully treated by the removal of definite foci of infection, elsewhere in the body. For instance, after the removal of abscessed teeth such a joint may show rapid improvement and ultimate recovery. Because of this curious relationship of joints showing sterile effusions to pyogenic foci elsewhere in the body, Joseph Freiberg[35] has previously designated them as "allergic joints."

In certain, and rather numerous instances, however, the exposure and removal of apparent foci does not result in a cure and it has been with these cases, alone, that we have been experimenting. All sources of chronic infections which have not been removed surgically have been carefully worked out from the bacterial standpoint. Old chronic sinuses and the lower bowel have most frequently yielded the organisms to which patients showed skin sensitivity and vaccines made from such organisms have been satisfactory in the treatment of many

of these cases. Certain patients in this series had been under treatment for years prior to an attempt at bacterial desensitization. Other forms of treatment were discontinued during the course of vaccine treatment.

The following table serves to summarize this group of patients and the results obtained.

Table V.

Case	Duration	Parts Involved	Focus	Organisms Sensitive	Result
1	3 years	right knee and elbow	Sinus	Streptococcus, hemolytic	Cured
2	18 months	knee and sacro-iliac	Sinus	Staphylococcus, hemolytic	Cured
3	14 years	both knees	Sinus	Streptococcus, non-hemolytic	Cured
4	9 years	both knees	Colon	Bacillus coli, Bacillus mucosus capsulatus	Cured
5	2 years	both knees and ankles	Throat	Streptococcus, green coloration	Cured
6	4 years	both ankles	Sinuses	Streptococcus, non-hemolytic Bacillus of Friedländer	Cured
7	6 months (flu)	right knee and right elbow	Ethmoid	Streptococcus, green coloration	Cured
8	3 years	right knee	Colon	Streptococcus, hemolytic Bacillus coli	Marked improvement
9	6 years	spine and both ankles	Colon	Bacillus coli, hemolytic (only)	Marked improvement
10	2 years	spine and elbow	Antrum	Bacillus coli, Streptococcus, hemolytic.	No improvement
11	4 years	knees	Colon	Bacillus coli, hemolytic	No improvement
12	5 years	Wrist, elbows and knees	Antrum and Ethmoid	Staphylococcus, hemolytic; Streptococcus, green coloration.	Marked improvement

From the table it is seen that 10 of 12 cases either been cured or definitely improved. Only two do not show improvement. Surely this seems to fix this group as an entity definitely benefitted by bacterial desensitization.

Several cases of arthritis deformans have been treated with mixed vaccines composed of those organisms recovered from such sources, to which they are definitely sensitive. The colon bacillus is a frequent offender, and is sometimes obtained from the stool in pure culture. This group is hard to follow since various other forms of treatment are carried on simultaneously, - the prime consideration being the alleviation of pain. Progress is very slow but in some instances it seems that the pain has been relieved and further progress of the disease impeded. "Cures" are out of the question - and we are not yet entirely satisfied that we have helped any case of arthritis which does not fit into Freiberg's classification of "allergic arthritis."

D. Angioneurotic Oedema.

Angioneurotic oedema is a rare and curicus disease. At the present time there is no generally accepted opinion regarding its cause or the mechanism by which the symptoms are produced. Clinically it is characterized by sudden, acute, swelling of various portions of the body as for example, a hand, cheek, the entire face, etc. Death has occasionally followed acute swelling of the larynx. Such patients frequently give a history of transient asthma, allergic joints or hives and these associations have led us to suspect that the condition may be a manifestation of bacterial sensitization.

Many investigators have considered the question of sensitization to foods and pollens but their results at desensitization have not been conclusive. Wherry [24] in 1927 reported a case of angioneurotic oedema which had been treated with marked improvement using an antigen of intestinal organisms to which she showed marked sensitivity. Unfortunately this patient died of an acute infection before he could come to any definite conclusion.

We have had an opportunity to study and treat a very typical case. The results justify its place in this thesis.

Our patient was a white woman, 40 years old who presented typical symptoms of swelling of the hands, feet and face which came on rapidly, lasted about twenty-four hours, and then disappeared as suddenly. These areas of swellings were definite, the same part of the hand, for instance, would swell repeatedly. Previous to the actual swelling she would notice heat and itching in the particular area where the swelling appeared.

This patient gave a history of a temporary asthma following influenza in 1918, which had been self-limiting. She also complained of gastro-intestinal symptoms and arthritic pains in the knees. She was given mineral oil to control the constipation. This present condition had been immediately precipitated by exposure to cold and water during the Florida hurricane of 1926.

She was found sensitive to eggs, milk, fish, and nuts, though she had never noted any relation between the eating of these foods and her attacks, and she was not relieved by eliminating these foods from her diet.

Stool specimens yielded a Staphylococcus aureus, Streptococcus hemolyticus and Bacillus coli communior. She was markedly sensitive to the Bacillus

coli communior. Treatment with this antigen was started in December, 1927. The first injections caused typical skin lesions to appear, nevertheless, treatment was continued three times weekly in the Clinic. Increased dosage again brought on fresh manifestations after an intermission of three weeks. The process was a tedious one and at times apparently rather hopeless, but gradually the attacks diminished in frequency and severity. Oedema of the larynx necessitated hospitalization on two separate occasions but these attacks were controlled with ephedrine.

In June, 1928, a fresh specimen of stool yielded the same flora with similar sensitivity reactions. Consequently she was treated with a fresh vaccine of the same organism until October, 1928.

Again in October the same organism was recovered and used. Her attacks, though much less severe and less frequent, still occurred at intervals during the fall of 1928. Treatment was continued until August 1929. At that time she was discharged and told to return in the event of a recurrence of her former trouble.

For a year and a half now she has had no recurrence of her angioneurotic symptoms. This offers an entirely new method of treating cases of angio-

neurotic oedema and a new point of view regarding the probable cause. It also explains the curious familial history long recognized as one of the peculiarities of the picture. We have come to expect family histories from patients who show these unusual types of sensitization.

E. Skin Infections due to the Staphylococcus, the Streptococcus and the Bacillus acne.

This group consists mainly of cases of furunculosis but there are included cases of streptococcus skin infection, staphylococcus osteomyelitis, one of a staphylococcus septicemia, and acne due to the Bacillus acne. As noted in the introduction, the treatment of furunculosis was the first digression of Wright from the treatment of typhoid fever.

It is well to review, in this connection, the work of Besredka with anthrax and later with staphylococcus and streptococcus infections.

Previous to 1925, Besredka had shown, to his own satisfaction, that in the case of anthrax the susceptibility of an animal is limited to the cells of the skin, exclusively, and that no immunity is conferred except by vaccination of the receptive cells with attenuated organisms; that is, entodermal

vaccination.

Staphylococci and streptococci, likewise, show a predilection for the cells of the skin. Attempts to demonstrate antibodies, of the known varities, have been universally unsuccessful. However, the success of vaccine therapy in such cases, indicates some other form of immunity. In fact we recognize an immunity, acquired during the course of the infection, in its self-limiting nature. Such immunity we observe to be transient; reinfections may occur whenever the general resistance is slightly lowered.

Besredka believes that the logical route of elective vaccination is the ordinary route of infection. The reticulo-endothelial cells in the skin are probably the receptive cells. He applied his results with anthrax vaccination to the treatment of staphylococcus and streptococcus infections, by vaccinating the receptive cells. He states that intradermal injections are much more effective than subcutaneous injections in treating skin infections. The immunity conferred, though it be limited to the skin, serves to protect the host from internal infection, by virtue of his skin immunity. Infections of internal organs are usually known to be preceded by infections of the skin or mucous membranes covering the surfaces of the body.

One aims to protect the healthy cells surrounding the localized infection and keep them from becoming infected, thereby favoring localization. Such a process may be a slow one and is usually aided by surgical removal of the degenerated tissue. This cuts down absorption and assists in maintaining circulation in the parts cut off by oedema. If localization of the infection is not accomplished, then a blood-stream invasion, by way of the lymphatics, may result. Actual growth in the blood stream is unusual but invasion of the blood stream is followed by secondary localization in the skin or in the parenchyma of the internal organs.

Streptococci localize in about the same manner, but tend to invade the blood stream more frequently than staphylococci. Vaccine therapy for localized infections due to the staphylococcus has been employed since Wright but recently has fallen into disfavor. It is less often used for treating streptococcus infections since further invasion is frequent and too rapid to be benefitted by such therapy. Those cases which tend to becme chronic streptococcus infections may often be controlled with vaccine. Generalized infections are not usually so treated, since ill effects have been often observed. However, we have

found that in some instances, vaccines given in minute amounts brought about rapid clinical improvement even in actual septicemia.

As a general rule, patients with local skin infections do not show the marked skin sensitivity evidenced in many of the groups previously described. This is undoubtedly due to the fact that constant autodesensitization of the ectoderm occurs due actually to the selective localization of the invading organism.

In brief our results have been similar to those of Besredka. We have found that entodermal injections are far more effective than subcutaneous injections in the vaccine treatment of skin lesions. The desensitization method of treatment is more effective than the "massive dose" method, the latter, in fact, is definitely contra-indicated. We also believe that intradermal desensitization favors the localization of skin lesions and inhibits farther spreading. Acne lesions seem to respond to treatment more rapidly when alternate intradermal and subcutaneous injections are employed.

The following table will serve to summarize this series of cases:

Table VI

Case	Diagnosis	Organism	Test	Treatment		Result
1	Furunculosis	Staphylococcus aureus	+	Intradermal	2 mos.	Cured
2.	Injury Furunculosis	Staphylococcus Aureus	+	Intradermal	1 mo.	Cured
3	Furunculosis	Staphylococcus aureus	±	Intradermal	1 mo.	Cured
4	Furunculosis	Staphylococcus aureus	+	Intradermal	1 mo.	Cured
5	Furunculosis	Staphylococcus aureus	++	Intradermal	1 mo.	Marked improvement
6	Furunculosis	Staphylococcus aureus	++	Intradermal	Irregular	Recurrence
7	Furunculosis	Staphylococcus aureus	+++	Intradermal	3 mos.	Cured
8	Furunculosis Abscess	Staphylococcus albus	++	Intradermal	1 mo.	Marked improvement
9	Furunculosis Abscess	Staphylococcus aureus	++	Intradermal	1 mo.	Marked improvement
10	Furunculosis Carbuncle	Staphylococcus aureus	++	Intradermal Subcutaneous	3 wks.	Cured
11	Furunculosis Carbuncle	Staphylococcus aureus	+	Intradermal Subcutaneous	3 mos.	Improved
12	Diabetes Furunculosis Carbuncle	Staphylococcus aureus	++++	Intradermal Subcutaneous	1 mo.	Healing Accelerated
13	Acne Osteomyelitis	Staphylococcus albus, Staphylococcus aureus	+ +	Intradermal Subcutaneous	2 wks.	Healing Accelerated
14	Furunculosis Septicemia	Staphylococcus aureus hemolytic	+	Intradermal Subcutaneous	2 mos.	Cured
15	Acne	Staphylococcus albus Bacillus acne	+	Intradermal Subcutaneous	1 yr.	Improved
16	Skin infection of foot Lymphangitis	Staphylococcus albus, hemolytic Streptococcus hemolyticus	- ++++	Intradermal Subcutaneous	2 mos.	Cured
17	Acne	Acne bacillus	++	Intradermal Subcutaneous	6 mos.	Cured
18	Acne	Acne Bacillus	++	Intradermal Subcutaneous	4 mos.	Markedly improved
19	Acne	Acne bacillus Hemolytic Staphylococcus	+ +++	Intradermal	3 mos.	No improvement

F. Non-ulcerative Colitis

This last group of patients studied does not constitute a definite clinical entity. The symptomology is varied and such diagnoses as mucous colitis, spastic colitis, the irritable colon and gastro-enteric neurosis are commonly used to give a pseudo-scientific name to the patient's complaints. It must be agreed that many factors other than disease of the colon itself come to play a large part in the production of the complete clinical picture. In some cases the neurogenic element will predominate and in others alterations in metabolism and endocrine disbalance may play a large role. We have tried to find a common cause which might be ultimately responsible for the varied symptomology such patients present.

Wherry [24] had previously shown that patients with mucous colitis are frequently extremely sensitive to certain organisms in their own intestinal flora and we decided to extend his experiments to include the whole group which we have just described. There is one physical alteration which is common to the entire group - namely, a spasm of the distal portion of the colon. It occurred to us that this spasm might be analogous to the spasm of bronchiolar hypertonus and that the entire picture might, in a mechanical sense be similar to asthma. The fact that mucous shreds from patients with non-ulcerative colitis show the same predominance of eosino-

philes observed in the sputum of asthmatics encouraged our belief in such an analogy. Therefore, we investigated the intestinal flora and hoped to identify certain organisms with the clinical problem on the basis of intradermal reactions.

Our earlier experiments were concerned with an attempt to find some specific organism, perhaps a streptococcus to which such patients could be shown to be sensitized. Careful bacteriological studies of the intestinal flora were made using varied tensions and selective media after the methods previously described. The results were unsatisfactory for we could demonstrate no specific sensitivity to any of the more unusual strains recovered from the feces of our patients. We then directed our attention to the "normal flora" and found that patients with nonulcerative colitis almost always exhibit a marked sensitivity to one or more of the so-called "normal flora". Again we have a situation which suggests the relationship to asthma. In both asthmatics and colitis patients the sensitization demonstrated does not involve the unusual, recognized, pathogenic types, but instead, the positive reactions are given by organism always found in the intestinal tract or the upper respiratory tract and which, too frequently, have been completely disregarded in bacteriological studies. Often times the active strains will demonstrate unusual qualities as, for example,

marked hemolytic power and frequently the small amount of antigen employed in carrying out the skin tests will precipitate a specific attack with all the symptoms common in a given case.

Strains of B. coli gave the most frequent reactions but B. mucosus capsulatus, B. fecalis alkaligines, and often B. cloacae and Proteus were active, in giving positive skin tests, in the order named. Many of these patients who had a history of long standing symptoms gave exceedingly severe reactions and it was not an uncommon thing to have the temperature reach 101 or 102 with adenopathy of the regional lymph glands and marked prostration. It has been our repeated observation that the more grave the patient's symptoms, the more marked will be the skin sensitivity and the more profound the general readtion.

We have attempted to treat a large series of patients with non-ulcerative colitis by desensitizing them with antigens composed of autogenous strains of organisms to which they have shown sensitivity. More than forty such cases have been carefully followed over a period extending from one to two years. We have found such desensitization most difficult to accomplish. It is always necessary to proceed very slowly in order to avoid unfavorable reactions but on the whole, the results have been encouraging. The criteria of cure or even improve-

ment in this group are somewhat uncertain because of the complicating factors we have already mentioned but in the face of such difficulties many of our patients have shown lasting and pronounced benefit following treatment. This problem is so large and so involved that we can do no more than introduce the subject in this thesis. Several years of observation and treatment are ahead of us before we can come to definite conclusions but we have established several significant facts: First, that this group of patients are invariably sensitized to certain of the so-called "normal flora", second, that the active strains when injected produce symptoms similar to those characteristic of the patients attacks and third, that desensitization with vaccine prepared from the active strains has been accompanied by marked clinical improvement in a large group of cases.

IV. Summary.

As stated in the introduction we undertook this study with the purpose of submitting to critical investigation the effectiveness of vaccine therapy when controlled by the intradermal test. We have presented data obtained from the study and treatment of six conditions, each constituting a well recognized clinical picture. In each group the number has been small enough to permit intensive and individual study, and large enough, at the same time, to allow us to come to definite conclusions. In all we have included 110 cases, none of which has been followed less than six months since the institution of treatment.

We believe that we have demonstrated beyond a question of doubt that vaccine therapy, as controlled by the intradermal test, is of great importance in biological therapeutics and that we have explained, in part, the loss of interest in such treatment. Our conclusions may be summarized under the following heads:

1. Specificity of bacterial vaccine therapy and selection of antigens by means of the intradermal test.
2. Method of treatment.
3. Reasons for failure of previous bacterial vaccine therapy.

1. Specificity of bacterial vaccine therapy and the selection of antigens by means of the intradermal test. Thruout our work we have been confronted constantly with one dominant fact, the essential specificity of bacterial vaccine therapy. Two factors are involved in each instance, the host and the offending organism, and the relation which exists between the two appears to be specific in a strict sense. The fact that an organism falls into a definitely recognized classification, bacteriologically, does not serve to indicate its rôle in a specific clinical case. For instance, S--- may have a staphylococcus in pure culture in his ethmoid cells but it does not follow that treatment with any staphylococcus will aid in curing his infection. It must be the same staphylococcus which he harbors in the mucous membranes of his upper respiratory sinuses. Again, there may be other organisms in addition to the staphylococcus present, and easily recovered, but are merely saprophytes. The intradermal reactions serve to indicate the specific relationship which exists between the harmful organism and the host, and makes it possible to rule out the harmless strains. The facts have been adequately demonstrated by the experimental evidence offered in the body of the thesis. We feel that the first and most important requirement for adequate bacterial vaccine therapy is that the organism employed in the antigen be selected on the basis

of positive intradermal reactions. These organisms should, whenever possible, be of autogenous origin but should it be necessary to resort to the use of strains not autogenous, they too, must be selected on the same basis of the positive intradermal reaction.

2. Method of treatment. Bacterial vaccines may be employed for two reasons: to immunize a non-infected individual and to treat an already infected individual. We have been primarily interested in the second problem. Massive dosage has long been employed for the purpose of protecting a non-infected person and the prophylactic use of typhoid vaccine is an example of the success of such a procedure. It was natural that the use of large doses would be carried over into the other phase, that of treating already infected patients. We have repeatedly indicated in our experiments both the error and the danger of such treatment. Desentization of sensitized patients, employing very small doses has given excellent results, whereas the use of massive dosage has usually resulted in exaggeration of symptoms.

3. Reasons for failure of previous bacterial vaccine therapy. We conclude that much of the disappointment attending vaccine therapy has been due to a failure to recognize the factors just discussed: first, that antigens should be selected on the basis of specific reactions to intradermal injections and second, that persons so shown to be sensitized should be desensitized by injections of very small amounts over a

long period of time.

Stock vaccines have been inadequate because the fundamental factor of specificity was entirely overlooked and, very frequently the stock vaccine has been given only to result in the sensitization of the individual to strains previously biologically inert. It must be remembered that an individual who receives injections of antigens, to which he has not been previously sensitized, will, as a result, become subsequently sensitized to those same organisms. This will probably happen when large amounts are injected and such has been the routine procedure in using commercial stock vaccines. This fact has been demonstrated on numerous occasions.

When autogenous vaccines have been used, it has been the custom to make the antigen from the prevailing strains. Such a practice should, on the surface, appear absurd for it is most frequently the harmless saprophyte which predominates on culture; yet this has been for years the last word in "scientific technique".

It is small wonder that bacterial vaccine therapy has fallen into disfavor.

BIBLIOGRAPHY

1. Wright, A. E., Lancet, Lond., 1900 - 1907
   Brit. Med. Journ., 1900 - 1907

2. Metchnikoff, "L, Immunite' dans les malades infectieuses".

3. Denys et Leclef, La cellule, 1895, xi.

4. Bulloch, W. M., Practioner, Lond., 1905, lxxv, 589.

5. Mathews, J., Lancet, Lond. 1908, 11, 925.

6. Symposium on Vaccine Therapy, Trans. Ass. Am. Physicians, Phil., 1907, xxii

7. Sherman, G. H., N.Y. M.J., 1910, xci, 1049.

8. Hektoen, Ludwig, and Irons, J. A.M.A., 1929, 92, 864.

9. Brieger, Ztschr. f. Hyg., 1895, 101.

10. Von Behring and Kitashima, Berl. klin. Wchn., 1901, xxxviii, 157.

11. Hericourt and Richet, Compt. rend. Soc. de Biol., 1898, 53.

12. Richet and Portier, Compt. rend. Soc. de Biol.
    1902, liv, 170
    1903, lv, 246
    1904, lvi, 302
    1905, lviii, 112
    1907, lxii, 358, 643
    1909, lxvi, 763, 810, 1005.

    Richet; Ann. de l'Inst. Pasteur,
    1907, xxi, 497
    1908, xxii, 465

13. Arthus, Comptes rend. Soc. de Biol., 1903, lv, 817

14. Von Pirquet and Schick, Wein. klin. Wchn., 1903, xvi, 758, 1244.
    Archiv. Int. Med., 1911, vii, 259, 383

15 a. Smith, Theobald, Jour. Med. Res., 1904.

15 b. Otto, von Leuthold Gedenkschrift, 1905, i.

16. Rosenau and Anderson, Bull. 29 and 36, Hyg. Lab., U.S.P.H. and M.H.S., 1906, 1907.

17. Besredka and Steinhardt, Ann. de l' Inst. Pasteur, 1907, xxi, 117, 384.

18. Besredka, Anaphylaxis and Antianaphylaxis, Gloyne, C. V. Mosby Co., St. Louis, 1919.

19. Zinsser and Parker, Journ. Exp. Med., 1917, xxvi, 411.

20. Kraus and Doerr, Wien, klin. Woch., 1908, xxi, 1008.

21. Delanoe, Comptes rend. Soc. de Biol., 1909, lxvi, 207, 248, 252, 389.

22. Holobuth, Zeitschr. f. Immunitätsf., 1909, 1, Orig. 111, 639.

23. Kraus and Amiradzibi, Zeitschr. f. Immunitätsf., 1910, 1, Orig. IV, 607.

24. Wherry, W. B., J. Infect. Dis., 1927, xli, 177.

25. Fischer, M. H. Soaps and Proteins, 1921.

26. Dorst and Wherry, O. St. Med. Journ., 1928, July.

27. Muer and Harris, Amer. J. of Publ Health, 1920, x, 874.

28. Stitt, Practical Bacteriology, Blood Work and Animal Parasitology, 7th Ed., 1923.

29. Rockwell, J. Inf. Dis., 1923, xxxii, 98.

30. Auer and Lewis, Journ. Exp. Med., 1910, xii, 151.

31. Walker, I. C., Boston M. & S. J., 1918, 179, 288

32. Lambright, from Dorst and Wherry, Ohio St. Med. Journ., 1928, July.

33. Kolmer, Infection, Immunity and Biologic Therapy, 1925.

34. Coca, Essentials of Immunology, 1925.

35. Freiberg, J. A., Arch. Surg., 1929, 18, 645.

36. Morris and Dorst, Bacterial Hypersensitivity of the Intestinal Tract, Unpublished.

37. Besredka, Local Immunization, 1927.