A CORRELATION OF THE TYPES OF SKIN REACTIONS WITH SPECIES OF STREPTOCOCCUS ISOLATED FROM ALLERGIC INDIVIDUALS

BY

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A THESIS

Presented to the Department of Bacteriology
and the Graduate Division of the University of Oregon Medical School
in partial fulfillment
of the requirements for the degree of
Master of Arts

June 1941

APPROVED:

[redacted]

(Adviser for the thesis)

[redacted]

(For the Committee)

May 15, 1941

PREFACE

This paper is devoted to the classification and identification of streptococci isolated from allergic individuals and furthermore, to correlate different types of skin reactions with the species of streptococci.

This work would not have been possible without the kind and sincere guidance and counsel of Dr. H. J. Sears, under whom I had the pleasure and privilege of working. I am also indebted to Dr. Sears for learned criticism and correction of this paper;; to Lucille Kellner for her very kind and careful proof reading; and to the cooperative staff of the Allergy Clinic for specimens.

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I. INTRODUCTION TO ALLERGY AND ANAPHYLAXIS

Allergy (ergia, "reactivity" and allos, "altered") is a term introduced by Von Pirquet, which Doerr adopted to signify any degree of specifically altered reactivity increased or decreased from the usual conditions, and depending upon the antigen-antibody reactions or upon the effects of entirely non-antigenic substances, such as hypersusceptibility to light and drugs. Allergy is usually spoken of as a condition unusual or exaggerated specific susceptibility to a substance which is harmless in equal amounts for the majority of the members of the same species. The term allergy is now used in a rather irregular and loose way. The present tendency is to classify allergy under a more general term, hypersensitiveness, which in the technical sense means an increased specific reaction capacity in an individual, man or animal, to a substance which in a normal individual of the same species, produces little or no reaction. It is evident that the manifestations of hypersensitiveness are varied and include such widely separated phenomena as the protein hypersensitiveness of animals, spoken of as anaphylaxis, and the allergies of man as hayfever, food, bacterial, and drug idiosyncrasies. Technically, all phenomena of specific hypersensitiveness are thought to be essentially immunologic in nature; thus it may be said that any classification of this nature is purely one of convenience for discussion. For seemingly all specific hypersensitiveness is the result of an appearance in the sensitized body of

a specific reaction product, a true antibody or its analogue, which, first, is incited by the contact of the cells with incitant and, secondly, in the mechanism of manifestation, mediates between the incitant and the cells, rendering them more than normally sensitive or capable of abnormal reaction.

Even at present these statements can be made only with reservation for in certain types of hypersensitiveness the mechanism is not well understood. There is one type of hypersensitiveness in which an antibody mechanism has been plainly demonstrated by another type in which, while remaining of specifically increased reaction capacity for an antigenic substance or for partial antigens, occurs without any definite evidence of the presence of the circulating antibodies. The statement has been made that basically, the mechanism of hypersensitiveness in man and animal may be due to the same immunological mechanism. The correlation of the human hypersensitiveness with the protein anaphylaxis in animals is obscured by the difficulty of determining the observed discrepancies. Whether the observed discrepancies were due to the fundamental differences of the mechanism or whether they are dependent upon the species' peculiarities of the anatomical structures or the physiological functions, is not yet certain.

Observations show that the conditions governing sensitization and the nature of response vary considerably with the indi-

vidual animal species. In human forms of hypersensitiveness, many are incited by true protein antigens; it seems apparent that the basic mechanism is one analogous to protein anaphylaxis of animals. Whether the evidence of human hypersensitiveness and the animal hypersensitiveness of anaphylaxis may be attributed to the manifestations of basically the same mechanism; it remains to be seen. The quantitative experiments, as conducted in lower animals, have not been extensively performed in human beings, which may account for the lack of extensive experimental evidences correlating human hypersensitiveness with protein anaphlaxis or animal hypersensitiveness.

In early experimental work with the animals, Wells (1921) laid down certain criteria which must be met in a condition to be regarded as a true anaphylaxis. It is well to review these criteria for as the experimental evidences are discovered in human hypersensitiveness; they seem to be closely related to animal anaphylaxis, if not the same manifestation of the basic mechanism. The criteria are as follows:

> "(1) The observed toxicity of the injected material must depend upon the sensitization of the animal, i.e., the substance must not produce similar symptoms in non-sensitized animals.
>
> (2) The symptoms produced must be those characteristic of anaphylactic intoxication as observed in the usual reactions with typical soluable proteins, being, therefore, the same for all antigens with the same test animals, but differing characteristically with each species of animals.

(3) It should be possible to demonstrate typical reactions in the non-striated muscle tissue of the sensitized animals.

(4) The possibility that the observed symptoms are caused by capillary thrombosis or embolism must be excluded.

(5) After the recovery from anaphylactic shock there should be exhibited a condition of specific desensitization to the same antigen under the proper conditions.

(6) In addition to the above, but not always, possible (a) to demonstrate passive sensitization with the serum of sensitized animals; and (b) to demonstrate amelioration or prevention of the bronchial spasm in guinea pigs by proper use of atropine and ephedrine."

It would be inadequate at present to base conclusions on criteria, for seemingly new experimental evidences are found which would necessitate broader interpretation or accepting those criteria as supplementary facts. It remains to be seen in the light of the future experiments whether human hypersensitiveness and the protein anaphylaxis of the lower animals are the same manifestations of the basic mechanism as presently thought.

The protein hypersensitiveness or anaphylaxis in animals has been thought as the reaction of an antigen with the tissue cells which have become sensitized from active treatment with antigen or serum containing specific antibodies. Whether an active or passive sensitization, it is thought that the reacting cells are associated with or contain specific antibodies which render them capable of abnormally rapid and excessive reaction with the

antigen when it reacts with the antibody in or upon the cell surface; consequently there is an injury of cells, an interference with the normal function,and perhaps causing an irritation, which are recognized by sudden or protracted anaphylactic shock.

With apparent similarity of the basic mechanism, no two species of animals display the same type of symptoms of hypersensitiveness.

The salient features differentiating the two conditions are the following: The exciting agent in anaphylaxis is always an antigenic substance; the exciting agent in human hypersensitiveness is often non-antigenic. Anaphylactic hypersensitiveness is induced by the injection of a sensitizing dose of antigen; hypersensitiveness in man often exhibited upon first contact with the exciting agent. The anaphylactic state, apart from congenital passive anaphylaxis, is not an inheritable condition; natural human hypersensitiveness shows a definite tendency to run in families, though the hypersensitiveness is often exhibited toward different substances in parents and offspring. Specific desensitization is far more easily demonstrated in anaphylaxis than in hypersensitiveness.

Acute anaphylaxis, natural hypersensitiveness, the allergic response, and specific antibacterial immunity appear to depend upon the fundamental mechanism, the union of the antigen with its specific antibody. The differences between them depend on the

nature of the antigen concerned, the nature of the animal into whose tissue it penetrates, the rate and the route of penetration, the distribution of the antibody in the animal's tissue, and the body fluids, the secondary effects of the primary cellular reactions, and a variety of other factors.

Although an extensive review of the literature may seem appropriate, a review will not be presented in the customary form. Since the subject of hypersensitiveness has been reviewed thoroughly by many workers, only attempts will be made to review briefly, in chronological order, that particular phase of the subject which seems to be most pertinent to the systematic development of the knowledge of hypersensitiveness. The chronological tabulation contains the essence of the stepping-stone experiments and the important conclusions, the only necessary observations which has directly advanced the theories of hypersensitiveness.

REVIEW OF OUR KNOWLEDGE OF HYPERSENSITIVITY

CHRONOLOGICALLY ARRANGED

Date	Nature of experiment and observation.	Significance and conclusion.
1565	Botallus states that smelling roses caused sneezing, and itching of nose.	Refraining from the use and contact of them prevented the symptoms.
1577	Von Helmont gives a description of seasonal asthma in a man and his mother.	He attaches no other significance than the detailed description.
1662	Schneider observed a peculiar reaction to rose pollen and wrote a detailed article on rose catarrh.	Made no conclusions concerning the nature of the reaction.
1673	Benningerus also made an observation on rose catarrh.	His article offered no essential explanation for the reaction.
1798	Jenner noticed an unusual reaction in second vaccination with small-pox vaccine material.	Explanation for the unusual reaction is not given.
1839	Magendie found that dogs which had been sensitized with egg albumin, upon reinjection died of shock.	Dogs repeatedly injected with egg albumin often died suddenly; he also gave a description of the symptoms.
1856	Blackley observed the reaction that he received from pollens and also observed the reaction on his patients; he reproduced typical symptoms on himself by application of pollens and pollen solutions to the mucous membranes of nose, eyes, and mouth. He also performed skin tests by applying small amounts of pollen on scarified area on the skin.	Statement is made that hayfever is caused exclusively by pollens; that symptoms of hayfever varied with the pollen content of the air.

1891	Koch observed that when a tuberculous patient had received tuberculin; he reacted in an unusual way which was characteristic.	Attributed the reaction to additional injury to the already injured tissue by the bacterial product (tuberculin).
1894	Flexner states that rabbits which withstood one dose of dog serum would succumb to a second dose if given after a lapse of some days or weeks, even though the second dose was sublethal for control animals.	Flexner made a careful observation, but gave no explanation for the mechanism.
1899	Bostock recognised hayfever as being caused by pollen.	Thought hayfever was caused by pollens and heat and sun's ray.
1902	Portier and Richet noticed that dogs which had been inoculated with eel serum became sensitive to their action so that second doses too small to be harmful to normal animals caused violent symptoms and death. Richet called this phenomena "anaphylaxis".	The reaction was attributed to manifestation of the animal to become without "protection".
1903	Dunbar confirmed the work of Blackley and isolated the active albuminous substance from the various pollens for treatment of patients.	Thought also that hayfever was caused by pollens in the air.
1903	Arthus and Breton found that repeated subcutaneous injections of horse serum in rabbits at intervals of several days gave rise to edema, sterile abscesses and gangrene at the site of innoculation.	Suggested that the condition was analogous to the experiments of Richet.
1904	Theobald Smith in standardizing the diphtheria antitoxin by the use of guinea pigs observed hypersensitiveness to second injection of antitoxin.	He made no conclusions.

1905	Otto made the first systematic study of anaphylaxis; found that an interval of 10 days elapsed after the sensitizing dose before hypersensitiveness was established; and that no hypersensitiveness resulted when large injections of serum were given at short intervals.	Attributed the toxicity of the second innoculation to the effect of the serum itself.
1906	Von Pirquet and Schick observed the first case of serum sickness due to second injection of diptheria toxin-antitoxin.	They attempted no explanation of the reaction theoretically.
1906	Wasserman and Bruck made the first suggestion of the immunological mechanism of the tuberculin reaction.	Attributed the reaction to harmful union of bacterial antigens and ambiceptors attached to the infected tissues.
1906	Wdff-Eisner observed series of hayfever cases and attributed the symptoms to the pollens.	Concluded that nasal pathology which occurred seasonally was due to hayfever caused by the pollens.
1906 1907	Rosenau and Anderson were the first to establish the specific nature of the anaphylactic reaction. 1. Guinea-pigs sensitized with horse serum showed no reaction to serum of other animals. 2. Single dose of horse serum which was harmless to normal guinea-pigs made the sensitized animal very reactive to second dose after an interval of 10 days. 3. The sensitizing dose was extremely small. 4. The hypersensitiveness	They concluded that "the fact that other animals besides man and the guinea-pigs react to a second injection of horse serum would seem to indicate that we are dealing with one and the same action". "It is probable that man may be rendered sensitive to the injection of a strange proteid. .".

	was lasting and also could be transmitted from mother to the offspring. 5. Found that sensitivity could be induced by the extracts of bacteria and believed that this may have a possible relationship to anaphylaxis and infectious diseases.	
1907	Otto made the first contribution toward the knowledge of desensitization. He found that animals recoverying from anaphylactic shock are desensitized for varying lengths of time.	He concluded that instead of death in properly desensitized animals after sufficient doses of antigen were given there was a period of condition in which ordinary doses could be tolerated.
1907	Besredka and Steinhardt made a more detailed study of the mechanism of desensitization. They found that sublethal doses of horse serum not only desensitized a sensitive animal but within a short time a high degree of tolerance was conferred; as much as one hundred to one thousand times the lethal dose.	They concluded that desensitization can be produced by the injection of sublethal doses of serum instead of production of a severe anaphylaxis.
1907	Nicolle published the earliest account of the passive transfer of the hypersensitive condition. He noticed that if a highly immunized rabbit's serum was injected into a normal rabbit, the injection of the original sensitizing serum rendered the rabbit reactive.	Nicolle concluded that the serum of the sensitized must contain a substance capable of responding to the horse serum.
1907	Gay and Southard are the first workers to support the	Their idea was based on the fact that the sensi-

	antigen-antibody theory of the anaphylactic reaction.	tizing fraction could be separated from the toxic fraction of the original antigen by ammonium sulfate precipitation.
1908	Nicolle and Abt attempted to disprove the work of Gay and Southard by using horse serum sensitized guinea-pigs.	They concluded that enzymes may be the cause of the anaphylactic mechanism instead of exotoxins or endotoxins. They determined the presence of antibodies in sensitized animals by compliment-fixation. Proved that sensitizing and toxogenic powers could not be separated.
1908-9	Victor Vaughan observed the so-called toxic and nontoxic fractions of tubercle colon, proteus, and other bacilli to study the reactivity upon injection into normal and infected guinea-pigs; also they observed hypersensitiveness of a person infected with micro-organisms to protein extracts of the infecting organisms.	Attributed the reaction to liberation of nonspecific poison by the parental digestion of proteins by specific enzymes developed under the conditions of the infecting organisms.
1909	Friedberger presented one of the earliest theories of the cellular cite of the anaphylactic reaction.	Thought that a precipitation reaction upon the surface of the tissue cells, loaded with receptors in the sense of Ehrlich represented the true mechanism of shock.
1909	Doerr and Russ using the serum of various animals made the greatest contribution toward the knowledge of passive transfer of sensitiveness. Hypersensitiveness to a given antigen can be pas-	Concluded that "the meeting of antigen and antibody in the blood stream alone could not account for the condition of hypersensitiveness.

	sively transferred not only by the serum of an actively sensitized animal but also by the serum of an animal in a state of desensitization or an anaphylaxis and even by the serum of an animal born congenitally sensitive.	
1909	Doerr shed the first probable explanation of drug idiosyncrasies; he found that addition of iodine or nitro group changed the specificity of the antigen.	Thought that the experiment helped to explain the specific nature of the anaphylactic reaction.
1910	Manwaring reenforced the theory of cellular site of reaction by exsanguinating a dog sensitized to horse serum and re-infused him with blood of a normal dog until there had been an almost complete replacement of blood; yet the dog remained sensitive.	"That the site of anaphylactic reaction does not take place wholly in the blood stream".
1910	Schultz contributed the incontrovertible proof of the cellular theory; he found that an isolated intestinal muscle of a sensitized guinea-pig in presence of a specific antigen would show contraction characteristic of anaphylactic reaction.	He concluded that since there was no blood in the isolated smooth muscle then the reaction must take place in the cells.
1910	Auer and Lewis published the first adequate account of physiological reaction that led to the death of an animal. They found that on post-mortem examination of guinea-pigs they died of anaphylactic shock distension of the lungs, due to the constriction of the bronchioles, which retained the air in the alveoli. Death was due to suffication.	The death of the guinea-pig was due to asphyxiation.

1916	Dale and Hartley confirmed the work of Rosenau and Anderson by using the euglobulin and albumin fraction of the horse serum; found that each fraction sensitized separately.	"The sensitization seems to be rigidly specific as when euglobulin produces sensitization to euglobulin, but not to pseudoglobulin.
1920	Coca was the first to make a thorough investigation on hypersensitiveness which he called "atopy". He contributed the differential characteristics between anaphylaxis in animals and hypersensitiveness in man.	He concluded that the following were the differential characteristics: 1. Exciting agent in anaphylactic shock is always an antigenic substance; while the exciting agent in human hypersensitiveness is often non-antigenic. 2. Anaphylaxis is induced by injection of an antigen; hypersensitiveness is often exhibited upon contact with the exciting agent. 3. Anaphylactic state is not inheritable; while human sensitiveness tends to run in families. 4. Specific desensitization is more easily demonstrated in anaphylaxis than in human hypersensitiveness.
1921	Prausnitz and Kustner record the first experimental study of passive sensitization in man. Kustner was sensitive to fish and found that his serum did not contain precipitation nor could they demonstrate passive sensitization of guinea-pig, but if small amount of his serum was injected into a normal person followed by in-	"Apparently the idiosyncratic serum contained something analogous to an antibody but probably not identical in every sense."

	jection of extra there was a marked reaction at the site of injection.	
1921	Wells established a group of criteria for anaphylactic reaction.	The criteria are as follows: 1. "The observed toxicity of the injected material must depend upon the sensitization of the animal, i.e., the substance must not produce similar symptoms in non-sensitized animals. 2. The symptoms produced must be those characteristic of anaphylactic intoxication as observed in the usual reactions with typical soluble proteins, but differing characteristically for each species of animals. 3. It should be possible to demonstrate typical reactions in the nonstriated muscle tissue of the sensitized animal. 4. The possibility that the observed symptoms are caused by capillary thrombosis must be excluded. 5. After the recovery from anaphylactic shock there should be exhibited a condition of specific desensitization to the same antigen under the proper conditions. 6. In addition to the above, it is usually, but not always possible (a) to show passive sensitization with the serum of the sensitized animal; (b) to show amelioration or prevention of the bronchial spasm in guinea-pigs by proper use of atropine and ephedrine.

1927	Best, Dudley, Dale, and Thorpe studied the effect of histamin on organs of the guinea-pig and attributed the shock to histamin; found that toxic syndrome produced by histamine reproduced very faithfully several of the characteristic features of acute anaphylaxis.	A sudden release of histamine in the circulation of an animal would have profound shock-like action.
1929	Avery and Tillett gave early account of bacterial haptens in anaphylaxis. They found that pneumococcal polysaccharide will not induce an active sensitization in guinea-pigs, but will induce an acute anaphylactic in animals passively sensitized by the injection of precipitating antisera prepared in the rabbit.	Concluded that the hapten fraction combined with the antibody produced in the rabbit previously and gave the reaction of anaphylaxis.

II. BACTERIAL HYPERSENSITIVENESS

As bacterial hypersensitiveness was studied in more detail, it became apparent that the mechanisms governing the anaphylactic reactions did not explain the tuberculin reaction, either in its systemic or in its local manifestations. It became clear that there were probably two distinct types of bacterial hypersensitiveness, one corresponding with considerable accuracy to protein anaphylaxis, the other, typified by reactions similar to tuberculin hypersusceptibility, which seems in many respects to differ from the anaphylactic phenomena. At present bacterial hypersensitiveness is discussed under two distinct headings: bacterial anaphylaxis and bacterial allergy.

Zinsser, 1921, found that the two types of hypersensitiveness, that is, typical anaphylaxis to tuberculous proteins and the characteristic tuberculin allergy could exist in guinea-pigs independent of each other or could be separately present in the same animal at different times. Guinea-pigs suffering from tuberculosis would give a marked skin reaction to O. T. 1 - 10 within ten days after innoculation without developing hypersensitiveness; while symptoms of anaphylaxis develop five to six weeks after the infection and sometimes does not develop at all. This important observation led to an attempt to separate in the tubercle bacillus the individual antigenic fractions responsible for each reaction, an effort which resulted in the dif-

ferentiation between nucleo-protein and a substance that Zinsser (1921) called the "residue antigen". Since antibodies could not be produced with these materials and yet they united with antibodies, a suggestion was made that they represented partial antigens or haptenes. A true anaphylaxis can be produced with tubercle bacillus material as with the antigenic substances of other bacteria, but animals so sensitized may not show any signs of that form of hypersensitiveness which is spoken of as allergy.

Bacterial anaphylaxis seems to be analogous in every respect to protein anaphylaxis, fulfilling all the criteria of Wells (1921). Sensitization takes place by antibody response to the whole or complete antigen in which nucleoprotein and carbohydrate fractions are combined. When such antibodies are sessile upon the cells, shock can be elicited in vivo and upon the isolated uterus with the specific carbohydrate substance alone.

Bacterial allergy is another form of hypersensitiveness which is not limited to the tuberculin reaction, but occurs in glanders and abortus infections. Allergy to bacteria may be described as a state of specific hypersensitivity to these microorganisms and their products which may be induced experimentally in animals and human beings or which occur spontaneously during the course of disease. During or after most infections an altered reactivity towards the products of the causitive agent of a disease may occur.

Koch (1891) contributed a basic observation leading to the recognition of bacterial allergy. Normal guinea-pigs may be injected with tuberculin in considerable amounts without causing noticeable symptoms. Tuberculous guinea-pigs react to comparatively small doses in a very characteristic manner. The fundamental characteristic in other tests of this nature seems to be the appearance of local and systemic reactions in infected subjects to contact with specific antigenic material which, in the same quantities, produce no effects in normal individuals. The likeness with the phenomena of anaphylaxis is thus indicated. The passive transfer which is characteristic of anaphylaxis has not been so far possible in tuberculin type of hypersensitiveness.

Zinsser (1921), in attempting to sensitize guinea-pigs with the nucleo-protein fraction of tubercle bacillus found tuberculin-like skin reactivity developed upon the use of unfiltered nucleo-protein. This observation led to further investigations. Tuberculin reactivity was demonstrated with dead tubercle bacilli as well as living, provided a tubercle had been formed. The filtered material did not produce allergy. This fact is important in demonstrating that the tuberculin reaction is not an infection reaction, possibly dependent upon the liberation from the living organisms of toxin or other products of secretion, but depends upon the formation of inflammatory tissue reaction.

These views were strengthened by subsequent studies on streptococcus and pneumococcus allergies. As a consequence the

allergic mechanism has been considered as a possible factor in the pathology of a number of important diseases of man.

Consideration of these few stated facts indicates that bacterial allergy is a specific hypersensitiveness to antigenic materials of the nucleo-protein type, which are discharged into the body from the bacterial foci where they are liberated by the action of the inflammatory tissue.

The role of bacteria in allergy had not been seriously considered until recently when certain conditions as asthma, rhinitis, migraine, urticaria, and angio-neurotic edema and other allergic conditions could not be explained on the basis of the reactions to ordinary food and inhalants. Walker and Adkinson (1919) and Hodge and Cohen (1921) reported finding predominence of certain types of streptococcus. Thomas, Famulener, and Touart (1923) confirmed the earlier results. The evidence strongly indicated that the causes of most of these allergic manifestations must be sought in the infectious processes within the body. Carmalt-Jones (1909) treated asthmatic patient with autogenous vaccine, first of the known records in literature. He resorted to autogenous vaccine treatment when he could not find the patient sensitive to the ordinary foods and inhalants. It is now generally accepted that the bacteria plays a certain role in allergic conditions where sensitization to food, inhalants, and contactants have not been found to be the responsible factor. Roger

(1921) and Vallery-Radot, Blamoutier, and Nitti (1936) found in allergic patients predominence of virilous streptococcus. The matter of disagreement concerns the question of specificity or non-specificity in the bacterial cases.

In bronchial asthma, it can be quite possible that the bacterial infection may by its presence in the bronchial tree, cause certain pathological changes to take place. The pathologically changed areas subsequently induce abnormal function of the organ which may be responsible for the observed symptoms in the asthmatics. In accordance with this, many authorities maintain that vaccine treatment is nothing more than foreign protein shock therapy (Rozendaal and Maytum, 1936; Feinberg, 1934; and Wilmer and Cobe, 1932). It is possible that any one or more of these non-specific chemical factors may be responsible for the relief in non-specific protein treatment. The pioneer research of V. C. Vaughan (1902) has indicated that any bacteria, including relatively avirulent B. Coli group, have inherent toxicity for the animal organism.

The opponents of non-specificity of bacterial allergy contend that specificity is demonstratable and that favorable relief depends upon specific reaction. This is characterized by those bacterial actions which are definitely sensitization phenomena.

At once when such a question arises, several difficulties

confront us. The supposed discrepancy between human allergy and animal anaphylaxis, owing to the frequent failure to obtain passive sensitization of animals by injection of allergic sera. But in spite of the known discrepancy, the majority of the investigators are inclined to agree with Zinsser (1926) that the basic principles of hypersensitiveness should not be neglected in favor of discrepancies which reveal lack of experimental knowledge, rather than fundamental differences of the principle. The fact that bacterial antigens and antibodies are extremely complex as compared with those of the simpler coagulable proteins, presents another formidable obstacle in the general agreement of the mechanism.

"Sensitization to bacterial antigens cannot, however, be produced with the same ease and certainty, as in the case of horse serum or egg-white. A single injection of bacteria or bacterial extract frequently fails to produce any demonstratable sensitization, and the most successful results have been obtained by those workers who have employed repeated small injections of antigen for sensitization, followed by an interval of three weeks or so." Topley and Wilson.

Bacterial anaphylaxis has been accepted and thoroughly established as a scientific fact. Rosenau and Anderson (1907) were able to induce active and passive sensitization with bacterial antigen. Zinsser and Parker (1917) established the bacterial anaphylaxis as a scientific fact by the Dale's - Schultz technic

with the isolated uterus. The reactions were demonstrated to be specific and desensitization can be brought about by the use of specific antigen.

The bacterial hypersensitiveness or bacterial allergy which concerns us in asthma and other allergic conditions is essentially different from the anaphylactic phenomena. Zinsser and Tamiya (1926) state that in the cases of bacterial hypersensitiveness that true protein anaphylaxis plays a relatively unimportant role and that the true mechanism is not well understood. The bacterial allergies or bacterial hypersensitiveness are of great importance since they develop rapidly and render the infected animal highly vulnerable to the products of bacterial growth, which are relatively innocuous for normal animals. They also believe that allergy is in no way related to that phase of resistence which is determined by the antibodies. Zinsser and Grinnell (1925), working with hemolytic streptococcus announced that there seems to be certain points of close analogy between streptococcus allergy and the tuberculin reaction. This observation is particularly important in that they believe all bacterial allergies are based on identical fundamental phenomena. To bring the tuberculin analogy closer, it was found that Dick filtrate, which corresponds in manner of production to an unconcentrated "Old tuberculin" without glycerine, is most potent material for demonstrating allergic state of skin reaction.

The bacterial allergy is usually dependent upon the presence of whole bacteria in the host and appears to be related to the nucleo-protein - carbohydrate combination of Avery, Zinsser, et al. This nucleo-protein and S.S.S. fraction suggests the probability of a multiplicity of specific soluble products of bacteria.

A brief analysis of the role of bacteria in allergic conditions is difficult to achieve chiefly due to the complexity of the bacterial effects in the human organism. These include not only the foreign protein factor, but the apparently more significant specific nucleo-protein - carbohydrate antigens, and are an entirely different hemolytic streptococcus factor of Sabins (1931) and Enders (1930), a true toxin which is heat resistant, but which incites a specific antitoxin. Any of these components of the bacteria may be present in various amounts; also they may be concerned with the allergic reactions of asthma, angioneurotic edema, rhinitis, and other allergic conditions.

These developments in bacterial allergy have been reviewed thoroughly by many workers. A brief review has been made chronologically to show the development of the knowledge of bacterial allergy and its possible significance to human disease. From these reviews it seems very probable that bacterial allergy has an important role in human disease.

CHRONOLOGICAL DEVELOPMENT OF BACTERIAL ALLERGY

Date	Nature of Experiment and Observation.	Significance and Conclusion.
1891	Koch showed that O.T. (old tuberculin) may be injected into a normal guinea pig in large amounts without causing noticeable effect, whereas a tuberculous guinea-pig reacted to a comparatively small dose.	A basic observation leading to recognition of bacterial allergy. Koch's conclusion: That tuberculin contains a substance responsible for the necrotizing action, which was particularly powerful upon tissues saturated with toxic substance.
1896	Babes and Broca, using tuberculin made similar observation to Koch.	They attributed the systemic symptoms to a sudden lighting up of the existing lesions by the tuberculin added to that already present.
1906	Wasserman and Bruck first suggested the immunological mechanism of the tuberculin reaction.	Tuberculin reaction was caused by the union of the tuberculin with the sessile receptors upon the diseased tissues.
1907	Von Pirquet, using the tuberculin, introduced skin test and also a term allergy or altered reaction capacity.	He gave no basic theories of the mechanism of altered reaction.
1907	Rosenau and Anderson, using extracts of tubercle, colon, typhoid, and anthrax bacilli observed one of the earliest anaphylactic reactions to bacterial extracts which resembles serum anaphylaxis.	From the result of the experiment, suggested that the incubation period for many of the infectious diseases represented the time necessary for the development of sensitization.

1909	Bail attempted a passive transfer of tuberculin reaction using mash of tuberculous organ; the guinea-pigs injected with finely divided material intraperitoneally died when they were injected 24 hours later with tuberculin	Concluded that passive transfer cannot be regarded as possessing character or regularity of passive transfer in anaphylaxis.
1910	Baldwin made the first theoretical explanation of the tuberculin reaction.	He showed that the skin sensitivity was produced only when actual infection had taken place; also that there seems to be a discrepancy between the skin sensitivity and general anaphylaxis.
1913	Weil showed that the horse serum which sensitized cells passively could be detected by the Dale method to be present as a foreign protein in the cells of the sensitized animals.	The cells of a given species may be capable of association with the globulin from one foreign species, but not with those of another species.
1921	Zinsser demonstrated that a guinea-pig sensitized with tubercle bacilli a true anaphylactic sensitivity as shown by the isolated uterus, and typical tuberculin reactivity of the skin could be separately present at different times in the same animal or could exist alone in a single animal.	Concluded that the tubercle bacillus was composed of at least two antigenic fractions which were responsible for the two reactions. Investigations led to differentiation between nucleo-protein and so-called residue antigen.
1923	Heidelberger and Avery purified the residue antigen of staphlococcus and other organisms.	They found it to be a carbohydrate substance which was type specific and was incapable of antibody formation, but combined with the antibody made by a complete antigen, the non-hydrolized organism.

1923	Zinsser and Parker obtained a definite anaphylactic reaction with pneumococcus extracts and isolated uteri of sensitized guinea-pig by the Dale method. Both active and passive sensitization was successful.	Led to the following conclusion: 1. That the so-called residue antigens were probably not protein and since antibodies could not be induced, they must be haptens. 2. That in the course of any infection in which bacterial foci are formed, there follows the development of a type hypersusceptibility which is distinct from protein anaphylaxis, which later were associated with the bacterial nucleo-proteins and which can be determined by intradermal skin reaction.
1925	Laidlaw and Dudley demonstrated with tubercle bacilli that the residue antigen which gave the skin reaction could be separated from that which was precipited by the anti-bacterial serum.	Separation of the two substances was thought to be fundamental to the understanding of the bacterial anaphylaxis.
1926	Long produced an active tuberculin reactivity in guinea-pigs by the injection of dead tubercle bacilli.	The mechanism opposing the super infection is essentially allergic; and that such allergy is means of attacking the specific invaders.
1927	Zinsser and Grinnell, by using pneumococcus autolysat, formulated on the basis of the experimental knowledge the probable mechanism of bacterial allergy.	1. The specificity of the tuberculin and similar reactions necessitate the assumption that the stimulating substances must emanate from the bacteria. 2. The development of allergy after the injection of dead tubercle

bacilli excludes the participation of a soluble or other substances produced by only the living organisms.

3. Allergy develops typically only when the tissue reaction has occurred around the injection, and it would therefore seem that the action of the inflammatory tissues, possibly enzymes, is necessary for the liberation of the sensitizing antigen from the bacteria.

4. Nucleo-protein that sensitizes to the tuberculin type of reaction cannot be obtained from the tubercle bacillus; in case of which severe and prolonged treatment is necessary, a fact which would indicate that test tube manifestation destroys the antigenic properties of something which is formed more gently by the action of the inflammatory tissues upon the bacteria.

5. With the organisms in which the nucleo-protein fraction can be obtained, without chemical manipulation by autolysis, typical tuberculin-like reaction can be induced by active sensitization.

III. STREPTOCOCCUS IN ALLERGY.

Its wide distribution and prevalence has placed this organism under suspicion in cases of allergy where evidences point toward bacteria as a probable cause. Walker and Adkinson (1919) made a thorough study of the groups of streptococci found in the sputums of bronchial asthmatics. They reported finding a predominence of the hemolytic variety, the alpha and gamma varieties in approximately equal numbers. In studying chronic non-tuberculous respiratory infections, Hodge and Cohen (1921) concluded that the flora of the sputum varied very little in asthmatics. They report that as many as eight to fourteen types of streptococci had been found in a single specimen.

A careful and thorough bacteriological study of the streptococcus in the sputum by Hooker and Anderson (1928) has revealed the predominence of the alpha type of Brown. It was found that the alpha type constituted seventy-six per cent, the beta type nine per cent, and the gamma nine per cent of all the organisms found in the sputum. These results were confirmed by Vallery-Radot, Blamoutier, and Nitti (1936). They reported predominence of the Viridans type of streptococcus in asthmatics and also in the cases of bronchitis. Bray (1937) reviews the role of bacteria in asthma and states that Wilmer and Cobe (1933), comparing the incidence of bacteria in 500 asthmatics and 200 normal controls, found that asthmatics showed the highest inci-

dence of streptococcus (67 per cent), while the normal control showed a predominence of staphylococcus (84 per cent).

The chief objective of this experimental work was the determination of the species of streptococci isolated from the asthmatics and further to correlate them with positive skin reactions and certain other phenomena exhibited by the patient as high eosinophilia of the blood and sputum. The streptococci were all isolated from sputum, stools, nasopharynx, and skin of allergic individuals in the Allergy Clinic. Almost exclusively the strains were isolated from bronchial asthmatics, but a few were from allergic rhinitis, eczema, and other conditions in which no appreciable sensitization was attributable to foods, inhalants, and contactant. The patients were routinely tested for sensitization to pollens, epidermals, foods, grasses, and other non-bacterial proteins. The cases showing negligible skin reactions to these were suspected of bacterial allergy. Sputum and stool specimens were taken routinely in suspected bacterial allergy cases, but frequently other specimens from the nasopharynx and from skin lesions were also taken for preparation of autogenous vaccines and filtrates to be used in the skin tests and desensitization treatments. The method of vaccine and filtrate preparation is described fully in the section on methods and technique.

The carefully prepared vaccines and filtrates were used in skin tests to determine the therapeutic dose of each preparation. For the skin tests, .02 cc. of the sterile, undiluted preparation

was injected intradermally into the inner aspect of the upper arm. The skin reactions were read in 15 minutes as immediate reactions and after 24 hours, as delayed reactions. The intensity of the skin reaction was primarly based upon the size of the wheal and the erythema. Wheals not exceeding 1 cm. in size were recorded - or (±) questionable, a 1 cm. wheal was regarded 1 plus (+), a 2cm. wheal 2 plus (++), a 3 cm. wheal 3 plus (+++), a 4 cm. wheal 4 plus (++++). Tests were made with the vaccines and the filtrates made from the three predominent types of streptococci from each specimen.

The size of the reaction to the skin test determined the relative amounts to be used in the desensitization treatment. The size of reaction indicated the degree of sensitization to the bacterial substances. Also, it has been thought that skin reaction to autogenous bacterial substances indicates specificity.

Benson (1932) believes that the immediate reaction of a large wheal and its surrounding erythema is of non-specific origin due to the foreign material in the mediums and purely toxic effect of the bacterial extracts. If this assumption is correct, it offers a possible source of error in reading the skin test reactions. If such readings were made without regard to the possible non-specific reaction, much confusion and misinterpretation could only be expected to follow. Benson further claims that in a great majority of cases, the medium produces an immediate intradermal reaction entirely

comparable to that with the corresponding whole vaccine. The exceptions to the previous statement that immediate reactions are non-specific reactions, are cited by Benson. He found that autogenous bacterial vaccines of viridans streptococcus prepared from the stool cultures gave immediate reactions with erythema and wheal on the allergic patient, while the same treatment on the normal control individual reacted only slightly. It is his belief that it is a specific reaction because the reaction upon the normal control was much smaller and that the patient responded favorably to the desensitization procedures.

Thomas, Famulener, and Touart (1923) found that the viridans streptococcus usually gave early skin reactions and the hemolytic variety next in order, but they claim that late or early reactions are considered to be of equal importance in therapeutic treatment. Brown (1925) felt that late or 24 hour reaction had a definite diagnostic value. W. T. Vaughan (1931) states, "A delayed positive reaction indicates not only actual sensitization, but also infection". Thomas and Touart (1932), who have made an extensive study of bacterial allergy, believe, as Coca, that the late skin reaction to bacterial antigen indicates a bacterial allergy. They also contend that the early and the late positive reactions are specific phenomena and are due to forms of hypersensitiveness. Dorst and Hopphan (1936) find that delayed reaction must indicate bacterial allergy.

Remember that positive skin test reactions may be specific or non-specific. If the positive skin reactions are specific, careful desensitization procedures will result in amelioration and relief from the allergic symptoms. But if the positive reaction is not due to a specific cause, desensitization procedures will not yield results similar to specific desensitization.

We are not attempting to discuss procedures for desensitization, but are merely pointing out that the possibilities of erroneous interpretations of the positive skin reactions are ever present. It is needless to stress the necessity of employing controls; the earlier experimental investigations prove that a good control is indispensible.

Positive skin reactions are definitely of value as evidence of allergic condition interpreted properly.

The importance of age as possibility in altering skin reactions must not be ignored. Brown (1925) believes that asthma beginning after the fortieth year is almost always of bacterial nature due to the probable lowered resistance. Coke (1927) claims that in his investigations of the various age groups responding to skin reactions, the skin reactivity decreased with advancing age. But Pinchess and Miller (1932) disagree with this.

Whatever significance age may have upon skin reactions, the possibility that it is a source of non-specific reaction must not be forgotten. Treut (1936) found that skin tests have little value

in determining what etiological members of the streptococci are present, and he found that allergics were much more sensitive than the non-allergic individuals, regardless of their age.

Many debating and conflicting articles are published concerning skin sensitivity.to the bacterial substances and the exact relationships. The question is: Is the reaction an indication of specificity or non-specificity?

Sicard (1917) treated asthmatics with autogenous streptococcus vaccines. He found that bacterial preparations showing pronounced local reactions to intradermal skin tests, proved to be most effective in the process of desensitization. Walker and Adkinson (1919) and Thomas, Famulener, and Touart (1923) also agree that the most satisfactory results were obtained in the allergic patients when autogenous bacterial substances were used for the treatment. W. T. Vaughan (1931) claims that bacterial allergy is a specific reaction and that specific desensitization identical to that of anaphylactic desensitization is possible. Benson (1932) reports that desensitization to streptococcus hypersensitiveness in allergic patients is specific in carefully selected cases. It is the opinion of Thomas and Touart (1932) that the late skin reaction to bacterial vaccine indicates bacterial allergy, also that both the immediate and delayed reactions are specific in their reaction. Dorst and Hopphan (1935) maintain that skin reactions and desensitization are specific, but emphasize that the treatment of allergic cases is an individual problem. Relief and cure

of allergic conditions, Vlevisaker (1937) writes, are due to desensitization, not to protein shock therapy.

But Wilmer and Cobe (1932) maintain that relief in the treatment of allergic conditions with streptococcus substances secured by a non-specific protein reaction exists. And Waldbott and Ascher feel that skin reactivity is purely a non-specific protective response of local nature. Feinberg and Rosendaal (1934) advocate non-specific reaction in effects produced by the autogenous vaccines.

Opinions favor the specific nature of skin test reactions and desensitization.

It is not our objective to attempt any further explanations or conclusions beyond the present beliefs. The present tendency of the investigators is to favor specificity of bacterial allergics, such as those found in cases of bronchial asthma, migraine, angioneurotic edema, rhinitis and other allergic conditions where the patient does not elicit any significant signs of sensitization to common foods, inhalants, and contactant proteins. This opposes earlier beliefs that bacterial allergy is non-specific and that desensitization is due primarily to non-specific shock therapy. This opposition is strengthened by present experimental and clinical observations of bacterial allergy which reinforce each other and verify the theory of specificity.

In view of the present theories, one can maintain that bacterial allergy is specific in selected cases. Selected cases means that the patients are carefully diagnosed to exclude any non-

bacterial sensitization, that the etiological agent be attributed to bacteria, and mainly that they respond to desensitization procedures with favorable results.

The skin reactions to the autogenous bacterial substances, as we have attempted to establish them, indicate specific manifestation of bacterial allergy. Investigations have found other manifestations are present with bacterial allergy. The most important one which we will discuss is the high eosinophilia of sputum and blood.

In the past eosinophilia was thought to have direct relationship with allergic conditions in general. It has definite diagnostic values in the determination of bacterial allergy when non-bacterial allergy and parasitic infestations have been ruled out. The value of eosinophilia as a definite diagnostic aid in bacterial allergy is not yet firmly established and accepted.

Sterling (1920) found that the average blood eosinophile per centage varies from 5 - 13. Brown (1925) does not believe that high eosinophile has much diagnostic significance. The reports that highest eosinophile counts have been found frequently in non-sensitive patients; and that a definitely asthmatic patient was found to be without eosinophiles, have been made by Brown. In 1926 Brown made another extensive report on the possible significance of eosinophilia in various sensitive patients. Asthmatics gave an average of 7 per cent blood eosinophiles; non-asthmatics gave a 2 per cent count. He concluded that blood and

sputum eosinophiles were parallel. He believes that eosinophiles have no effect upon sensitization. Coates and Ersener (1930) maintain that the eosinophiles were due to an irritative reaction rather than to the infective nature. Cooke (1932) believes that local tissue reactions are responsible for the eosinophilia. And Van Leewen (1932) does not accept the theory that eosinophiles have anything to do with allergic conditions. Thomas and Touart (1932) found eosinophilia with many of their allergic patients. Hume (1936) finds eosinophiles in the nasal secretions of great importance in the diagnosis of allergy. Hunt (1938) states that eosinophiles are quite common in bronchial asthmatics, an average count being about 5 per cent. In his opinion the eosinophile count in an individual is not a static condition but is subject to large variations. He also found that any differentiation between infective and non-infective asthma is impossible. We have a theory that there may be a correlation between high eosinophile count and skin reactions.

PART II

Experimental

I. Object of the experimental work.

As previously stated the main objective of this experimental work concerns the determination of the species of streptococcus isolated from the asthmatics and its correlation with skin reactions; and also the correlation of skin reactions to high eosinophile counts of the sputum and the blood. Our interest in the possible relationship between the species and the skin reactions was promoted by several factors: From a purely bacteriological standpoint, it is definitely valuable to know the bacteriology of the sputum, stools, nasopharynx, and skin of allergic individuals. Should the classification give a predominence of certain species, it would be a great diagnostic value to the clinicians. A correlation between the skin tests and the species would be a great impetus and value to the allergists. By far, these are not the most important values of such an experiment and moreover it would be an interesting scientific contribution and perhaps a confirmation of some earlier results.

II. Procedures

Although an extensive review of methods and techniques might appear appropriate, only reference to the original authors contributing to the particular method will be made. Since Sherman (1937) has written an extensive and a thorough review discussing and evaluating the various methods used in streptococci classification, we have made no attempts to review the vast amount of work that has been published. The methods of classification of streptococci used by Sherman (1937) were adopted and used in our laboratory; since the method excellently serves the important purpose of isolating the related streptococci into distinct and manageable groups.

All the strains of streptococci classified were isolated from specimens received at the Allergy Clinic of the University of Oregon Out-Patient Clinic. The specimens were submitted in sterile containers. With few variations, the method of isolation was similar in all cases.

Sputum specimens: Five-tenths cc. of the sputum was ground in a sterile mortar with sand and 4.5 cc of sterile saline, making a 1-10 dilution of the sputum. From subsequent dilutions of 1-100, 1-1,000, 1-10,000, pour plates were made using 0.5 cc of the diluted solution. 0.7 cc of oxalated human blood was added to the infusion agar in making the blood agar plates. Direct smears of the sputum were stained by the Wiber modification of Wright's

stain and 100 white cells were counted for the eosinophile per centage.

Stool specimens: A large loopful of stool was inoculated into 4.5 cc saline. This solution was considered a 1-10 dilution of the specimen. As with the sputum specimen, blood agar plates were made of the 1-100, 1-1,000, and 1-10,000 dilutions.

Nasopharynx Swabs, Skin Cultures, etc: The swab was placed in 4.5 cc saline. This solution was considered a 1-10 dilution. Blood agar plates were made of three dilutions, and the eosinophile per centage was determined in the nasopharynx specimen in the same manner as the sputum specimen.

The media used for the cultivation of pure cultures of the streptococci was infusion broth to which had been added 1% dextrose. A small piece of beef brain was placed in each tube, the broth was added and the whole sterilized at 15 pounds pressure for 30 minutes. The organisms grew luxuriantly in 24 hours' incubations. Three of the four isolated colonies of each variety of streptococci were selected from a plate of every specimen, inoculated into the brain broth tubes, and allowed to incubate 18 to 24 hours. Organisms from each specimen showing the same type of hemolysis, growth on broth and staining characteristics were considered to be of the same strain. Suspensions of similar streptococci were decanted into sterile centrifuge tubes and centrifuged. The supernatant solution in the centrifuge tube was decan-

ted and filtered through a Seitz filter. The filtrate was subsequently pipetted into a 10 cc sterile vaccine vial, which was then sealed with a sterile rubber stopper. The sediment remaining in the centrifuge tube was washed twice with sterile Coca's solution, and resuspended in 10 cc. of the buffered salt solution. This suspension was pipetted to a vaccine bottle, capped, and heated for two hours at 58° C. Both the filtrate and suspension were tested for sterility in broth tubes. In this manner a sterile broth-free suspension and broth filtrate were made for each variety of streptococci present in a single specimen.

The organisms obtained for classification and study were inoculated into 1.0 per cent dextrose veal infusion brain broth media (pH 7.4) and incubated for 18-24 hours at 37° C. Growth appeared in the enriched basic media as a flocculatant, homogenous suspension, or heavy precipitate in the bottom of the tube. Before preparing the dilutions for plate inoculation, the culture had been subjected to agitation which thoroughly dispersed the large aggregates of the organisms in the culture media.

The initial step in classification is performed on the blood agar plate. Most satisfactory results are obtained by using 0.7 ml. of rabbit blood with 0.3 per cent potassium oxalate anticoagulant. Care must be exercised to add blood and organisms only to previously melted veal infusion agar cooled to 45° C., for a higher temperature destroys the red corpuscles and less

heat resistant organisms. Following the addition of the blood and inoculation of the organisms, the tubes were carefully shaken and the contents poured into a sterile petre dish. The plates were gently whirled and allowed to remain approximately 10 minutes at room temperature before final incubation at 37° C. for 24-26 hours. Incubation of 18 to 24 hours at 37° C. yielded most satisfactory results; however, further incubation of 48 hours was frequently necessary for the slow growing strains.

The colonies on the plate were classified as producers of hemolysin, methemoglobin or non-producers of reaction upon blood.

The microscopic appearance (Brown, 1919) of hemolytic or beta colonies upon blood agar is characterized by a clear, or distinct, sharp hemolysis zone immediately surrounding an elliptical or round colony which upon refrigeration and further incubation does not produce the so-called "halo" or concentric ring formation.

Whereas the methemoglobin producers or the alpha types are usually recognized by a distinct and definite greenish zone immediately surrounding an oval or round colony which upon refrigeration produces the "halo" effect. The "halo" phenomena is characterized by a clear zone of hemolysis occasionally containing cell fragments encircling the methemoglobin zone. Further incubation of 18-24 hours will result in production of a green methoglobin area peripherally to the existing zone of hemolysis, producing the "halo" appearance. Several consecutive "halos" may be produced in this manner.

Following the isolation and classification upon blood

agar a most typical colony is removed and inoculated into 1.0 per cent dextrose veal infusion broth and incubated at 37° C. for 18 to 24 hours. If the Gram stain prepared from the suspension appears homogeneous and free from contaminants, they were considered satisfactory for further classification. The pure culture in 1.0 per cent dextrose veal infusion broth served as a source of inoculum for stock cultures and for classification.

The stock cultures were grown upon a commercial preparation, North's gelatin agar (Difco). Transplantation of strains were necessary every twenty to twenty-five days. Previous to retransplantation the slow growing strains were greatly increased in the ability to grow vigorously upon the solid media by inoculation from broth culture.

The classification of the organisms were promptly pursued as immediately as possible. The temperature tolerance tests, 30 minutes at 65° C. and 60° C. were begun first; since during the incubation other tests could be simultaneously performed without appreciable loss of time.

Heavy broth suspension containing approximately 500,000,000 organisms were inoculated into a previously heated basic (pH 7.4) veal infusion broth containing 0.0005 per cent acid fuchsin (1.0 per cent Andrades indicator) and 1.0 per cent dextrose which is utilized by the organism with the production of acid, and consequent lowering of hydrogen-ion concentration. The lowered hydro-

gen-ion concentration is indicated by appearance of color varying from pink to red due to acid hydrolysis of acid fuchsin. To insure an equal amount of heat treatment for each tube, broth heated to 65° C. or 60° C. were inoculated every minute until all of the tubes were inoculated. In this manner the second tube would be taken out one minute after the first tube, third tube would have been removed two minutes after the first tube, etc., and the remaining tubes were removed in a like manner. Each tube taken out of the broth after thirty minutes was promptly cooled to 37° C. and immediately incubated at 37° C. In all cases the growth of heat tolerant organisms appeared within forty-eight hours. Further incubation of five days produced no appreciable change in color of the indicator or the turbidity of the broth.

The ability of the organisms to grow in 10° C. and 45° C. was also performed in the media used for maximum temperature tolerance test. Inoculations were made into the media at room temperature and placed in respective temperatures. Those strains which would grow in 45° temperature, produced sufficient growth within 24 hours and in spite of further incubation no changes were apparent after forty-eight hours incubation. The growth at 10° C. required minimum of twenty days for appreciable growth and color change in the indicator. If no changes were apparent after 24 days of refrigeration the test was regarded as negative.

The test to determine the ability of the streptococci to

grow in 6.5 per cent NaCl, 0.1 per cent methylene blue, and high alkalinity, pH 9.6 were performed by inoculating the organisms into freshly prepared media at room temperature.

For the 6.5 per cent NaCl tolerance test, 8.0 ml. of 1.0 per cent dextrose veal infusion broth (pH 7.4) was added to 2.0 ml. of sterilized 30 per cent NaCl shortly before inoculation. Growth appeared within twenty-four hours. However, further incubation of twenty-four hours was frequently necessary.

The methylene blue tolerance test was prepared shortly before use by addition of 1.0 ml. of 1.0 per cent of sterile methylene blue to 9.0 ml. of sterilized skimmed milk. Inoculations were made at room temperature and incubated at 37° C. for twenty-four to forty-eight hours. The ability to reduce methylene blue milk within twenty-four hours was considered very strong reduction. Whereas equal reduction requiring seventy-two hours was considered weak in reducing ability. If no apparent changes were visible after seventy-two hours the test was regarded negative.

The media for pH 9.6 was prepared accurately by the use of glass electrode pH-meter just before the inoculation. To 9.0 ml. of 1.0 per cent lactose veal infusion broth sufficient sterile 1/N NaOH was added to give the reaction a pH of 9.6. The turbidity of the broth within forty-eight hours served as an index of positive growth.

The hydrolysis test of starch was performed in liquid media, containing veal infusion broth, and 0.1 per cent soluble

starch. The organisms were inoculated and incubated 37° C. for five days. One milliliter of Gram's iodine was placed in the media. One ml. of Gram's iodine was also placed in an uninoculated media. The test was recorded as positive if the color of the iodine treated media was lighter than the control tube, which was almost black.

An aesculin hydrolysis test was also performed in liquid media containing 0.1 per cent aesculin and 0.05 per cent ferric citrate. After the organisms were inoculated in the media, incubation of twenty-four hours at 37° C. was sufficient to indicate hydrolysis of aesculin. The formation of a dark brown or black color throughout the media by the combination of hydrolysed products, aesculin and ferric ions was positive indication of hydrolysis.

Stabs were made in gelatin media for gelatin liquifaction test. Incubation of twenty to twenty-five days at room temperature followed by liquifaction was regarded as positive.

Sterile skimmed milk inoculated with a heavy broth suspension gave coagulation without peptonisation regardless of length of incubation, coagulation and peptonisation, or no apparent reaction upon milk. Coagulation took place within twenty-four hours and if peptonisation was to be affected, the reaction was complete within seventy-two hours.

In order to minimize the hazard of contaminants in the tests, two broth cultures were made of each strain. Second broth

culture was employed in fermentation or acid production from various sugars. Approximately 5×10^8 organisms were inoculated to insure heavy growth. The media composed of basic (pH 7.4) Hartley broth containing 5×10^{-4} per cent acid fuchsin (1.0 per cent Andrades) proved quite satisfactory for fermentation media. Control tubes without the sugars were prepared to be inoculated for each test organism.

The sugars were sterilized in neutral solutions to minimize hydrolysis and added to previously sterilized Hartley broth in 0.5 per cent concentration for rare sugars and 1.0 per cent final concentration for less rare ones. Several days previous to classification, the concentrated sugar solutions were added to suitable concentrations of 90 ml. of Hartley broth followed by twenty-four hours incubation at 37° C. If no growth of contaminants or changes were apparent, the media was aseptically tubed and incubated again to insure perfect sterility. The sterile clear media was heavily inoculated and incubated at 37° C. for five days; although twenty-four hours to forty-eight hours were sufficient for indication of strong fermentation reaction.

Although there are varying degrees of fermentation, any degree of positive change was recorded as positive test. Frequently, the test or the "sugar" tubes contained sugar which gave a positive reaction, in which case comparison was made with the control tube and "sugar" tube. If the "sugar" tube gave deeper

color tint than the control, the test was regarded as positive.

These groups of tests were carried out in the first series immediately upon isolation of the strains. The second series of tests were composed of fibrinolysis test and Lancefield's precipitin test for the serological classification of the pyogenic streptococci. The cultures for the second series of tests were taken from stock cultures which were kept in refrigeration.

III. Methods and Techniques.

Hemolysis. The reactions of the streptococci upon blood were determined by the use of rabbit blood agar, a method introduced by Brown (1919). Instead of defibrinated horse blood, 0.3 per cent oxalated rabbit blood was used, because of greater convenience of obtaining fresh lots of blood and the reactions of the organisms upon the rabbit blood agar seemed to reproduce quite faithfully the reaction obtained upon the human or horse blood. The agar base was composed of veal infusion broth prepared in the usual manner. Inoculation was made upon cooling (12 ml.) of melted veal infusion agar to 45° C. and upon addition of 0.7 cc. of 0.3 per cent oxalated rabbit blood. By means of serial dilutions, 75 to 250 organisms were inoculated into the liquified blood agar, which was carefully poured into a sterile Petri dish and permitted to cool 10 minutes after gentle mixing.

The rate of growth of a particular strain determined the length of the incubation period. In most instances 24 hours' incubation at 37° C. was sufficient to yield differential characteristics. Preliminary readings were made immediately as alpha, beta, or gamma types. The criteria of Brown (1) was followed for determination of types of hemolysis.

The alpha colonies were surrounded by a definite area of greenish zone of methemoglobin. Under a low power of the microscope the greenish zone was less apparent, but in outer portions

of the zone there was possibly a slight zone hemolysis. Grossly to the eye the deep colonies appeared as small biconvex, greenish colonies, while the surface colonies were round, flatly glistening, and greenish by transmitted light. The alpha prime, methemoglobin producers, upon ice-chest storage of 24 hours produced a definite ring of hemolysis immediately out-side of the methemoglobin zone, thus concentric zones of hemolysis were produced by incubation and ice-chesting until several rings of hemolysis were produced.

The alpha prime type of Brown (1919) is a strain of streptococci resembling closely the beta type upon the blood agar. Grossly it has all the characteristics of a beta colony with a zone of apparently clear hemolysis immediately to the outer edge of the colony, but upon examination with the low power of the microscope, many discolored and fragmented cells are found most abundantly in the proximity of the peripheral edge of the colony, which presents a hazy appearance. Upon storage of 24 hours in the ice-chest, apparent concentric ring of hemolysis were observed; the initial zone of hemolysis was noticeably enlarged and had become rather diffuse.

The beta types were isolated upon the following criteria of Brown (1919):

This type of reaction upon blood agar may be described as a colony surrounded by a definitely clear, colorless zone of hem-

olysis. A microscopic examination reveals no fragments of partially discolored corpuscles in the area immediately surrounding the colony. The distinguishing characteristics appear much earlier than the alpha types and are quite definitely developed in 18 hours at 37° C. The colony has a greyish appearance by the transmitted or reflected light. The surface and the deep colonies alike are surrounded by characteristic zones of hemolysis.

Further incubation at 37° C. for 24 hours does not appreciably increase the area of hemolysis. A period of 24 hours refrigeration as one might expect, does not increase the area of hemolysis, nor does the second period of incubation substantially produce apparent changes in the hemolysis area.

The gamma types of appearance on blood agar are characterized by the following properties:

According to the accepted criteria of differentiation, the gamma type produces a growth of colonies within and on the blood agar plate without the production of any perceptible hemolysis or discoloration of the surrounding medium during the incubation or refrigeration period.

Aesculin Hydrolysis

The ability of the streptococci to hydrolyze aesculin was determined in the absence of bile salts by a modification of Weatherall and Dible's (1929) method.

A heavy broth suspension of 18 to 20 hours was inoculated into a broth containing 0.1 per cent aesculin, 0.05 per cent ferric citrate. Immediately after the inoculation the tubes were incubated at 37° C. for a period of 5 days; although twenty-four hours yielded sufficiently necessary reactions of the test. A formation of dark brown coloration throughout the media, due to the ferric salt of the hydrolysate aesculin, ferric aesculitin, was considered characteristic indication of aesculin hydrolysis. Any detectable discolorations varying from the control tube indicate hydrolysis or a positive reaction.

Starch Hydrolysis (II)

One tenth per cent soluble starch in veal infusion broth was used to determine the ability of the streptococci to hydrolyse starch. The starch broth media was inoculated heavily with a suspension of 18 to 20 hours culture and immediately incubated at 37° C. for five days which was sufficient incubation for the complete hydrolysis of the starch; frequently within 48 hours some strains were able to completely hydrolyse the starch. Five tenth ml. of 0.33 per cent iodine and 0.7 per cent potassium iodide in aqueous solution was placed in the broth media. After ten minutes, if no distinct or apparent reaction had taken place between the starch and the iodine, the positive reaction is clear and slightly reddish blue,or yields no reaction with the addition of the iodine,unlike the dark blue coloration of the control or a negative test.

Methylene Blue Tolerance (7)

The method introduced by Sherman and Albus (1918) proved to be the most satisfactory for the determination of the ability of the streptococci to grow in the presence of a final concentration of 0.1 per cent methylene blue. The media was prepared by adding 1.0 ml. of sterile 1.0 per cent medicinal methylene blue solution to 9.0 ml. of previously sterilized skimmed milk.

A heavy suspension of 18 to 20 hours broth culture served as an inoculum for the test. The positive results were apparent and recognizable usually at the end of 24 hours incubation with partial reduction the methylene blue accompanied by only coagulation of the milk or coagulation with subsequent proteolysis of the coagulum. Frequently, some strains required 48 to 72 hours incubation to yield an appreciable reduction. The indication of the positive test was determined by the presence of any apparent reduction of the methylene blue with coagulation or coagulation and liquifaction of the coagulum.

Maximum Heat Tolerance (5, 4, 14, 15)

Instead of following strictly the method of Sherman and Stark (1931), a slightly modified method was used for the thermal resistance determination. The original test used by Sherman and Stark (1931) consisted of inoculating previously heated tubes with 1.0 ml. of milk culture which had been grown for 2 days at 37° C. Viability and the apparent number of organisms were determined by plating upon the lactose agar plate after and previous to the inoculation at the respective temperatures. Basic (pH 7.4) veal infusion broth containing approximately 0.0005 per cent acid fuchsin (1.0) per cent Andrades) and 1.0 per cent dextrose replaced the milk media of the original test. Approximately 500,000,000 organisms were inoculated from a 24 hours veal infusion broth culture into a previously heated media, (60° and 65° C.) and after the inoculation it was incubated exactly 30 minutes at the desired temperature. In order to insure an equal temperature treatment, a tube was inoculated every minute until all were inoculated. In this manner the second test tube would have had the same amount of incubation one minute after the first tube had been incubated exactly 30 minutes and the third tube would have had the required amount of incubation 2 minutes after the first tube, and so on to the last tube. A care was exercised to minimize the differences of temperature on the surface of the media, due to the evaporation

by maintaining constantly the level of the hot water 5 to 6 mm. above the level of the media within the tube. The test tubes were promptly subjected to cooling after exactly 30 minutes of heating and placed in an incubator at 37° C. Reading of the results was possible after 24 hours, but 48 hours incubation seemed more satisfactory for the final determination.

A positive test was characterized by the change of the colorless media to various degrees of pink to red of the acid fuchsin; the living heat resistant orgamisms hydrolizing the dextrose to acid is responsible for the change in the indicator system.

Temperature Limits of Growth
10° C. and 45° C. (2,3)

The uses of the temperature limits of growth of the streptococci was introduced by Sherman and Albus (1918) and Sherman (1937). The original experiment at low temperatures of incubation was conducted by inoculating litmus milk with the cultures and incubating them at desired temperatures. Growth was determined by the presence or the absence of visible change in the litmus milk.

For this experiment, instead of the original litmus milk, 1.0 per cent dextrose veal infusion broth with 1.0 per cent Andrades indicator was used. Immediately previous to the inoculation the tubes of media were placed in 45° C. water bath and for the 10° C. growth test, the media was placed in the ice chest for 30 minutes before inoculation. Inoculations were made as rapidly as possible at the room temperature, followed immediately by placing them in their respective temperatures.

The reaction of the 45° C. test was apparent and visible at the end of 24 hours incubation. Usually an additional 24 to 48 hours incubation did not produce any appreciable change in the intensity of the indicator.

At 10° C. the growth required to produce appreciable changes in the indicator ranged from sixteen to twenty days. After refrigeration of 24 days the intensity of the indicator was not changed. The reactions were recorded as positive or negative after 24 days of refrigeration.

Sodium Chloride Tolerance (8)

The test to determine the ability of the streptococci to grow in the final concentration of 6.5 per cent sodium chloride (Sherman, 1921) gave consistent and satisfactory results when the media was prepared immediately before the inoculation. A final concentration of 6.5 per cent sodium chloride was obtained by the addition of 2 ml. of 30 per cent sterile sodium chloride solution to 8.0 ml. of 1.0 per cent dextrose infusion broth previously sterilized with 0.0005 per cent acid fuchsin (1.0 per cent Andrades).

The inoculation was made immediately with a heavy broth suspension of organisms grown 18 to 20 hours in veal dextrose infusion broth. The indication of growth was usually apparent at the end of 24 hours, but 48 hours incubation yielded much more satisfactory results. Although the reactions were seemingly complete at the end of 48 hours, the readings and final observations were not made until the end of four to five days. The tubes containing cloudy suspension or heavy precipitate accompanied by the changes of the indicator to various hues of pink to red were regarded as positive. A clear broth tube without an apparent change in the indicator was regarded as negative or absence of growth.

Proteolysis (6)

Sterilized skimmed milk heavily inoculated with 18 to 20 hours dextrose veal infusion broth culture and incubated at 37° C. for 48 hours yields satisfactory evidence of proteolysis and coagulation of milk proteins. Some stains were only able to coagulate the milk regardless of the length of the incubation. Others were able to coagulate and liquify the coagulum, while frequently some would not produce any apparent reactions upon the milk. The final recordings and readings were made after five days of incubation.

For gelatin liquifaction test large loopfuls of broth culture were stabbed into the solid gelatin media. The incubation was made at room temperature for 4-5 days. The gelatin media was liquified in the positive test.

The pH 9.6 Tolerance (8)

The method introduced by Sherman (1921) for the determination of high alkaline tolerance proved to be very satisfactory and effective in classification. To previously sterilised lactose veal infusion broth in 9.0 ml. quantities containing 0.0005 per cent acid fuchsin (1.0 per cent Andrades indicator), immediately before, use, sufficient I/N NaOH is added to give a reaction of pH 9.6. The initial volume of I/N NaOH to be added was determined by the glass electrode pH meter (Beckman). A new determination of the correct amount of I/N NaOH to be added for each test was necessary because the alkali invariably absorbed Co2 from the air, subsequently lowering the normality of the alkali. Thus, if the original volume was added the resulting pH would be lower than pH 9.6.

An inoculum of approximately 500,000,000, organisms from 18 to 20 hours dextrose veal infusion broth was added to the tube and immediately incubated at 37° C. for 48 hours. If no growth occurs at the end of 48 hours incubation period the test is recorded negative.

Fibrinolytic Activity (12,13)

The method introduced and used by Tillett and Garner (1933) was followed in the determination of the fibrinolytic activity.

Ten ml. of normal human blood was placed in a tube containing 0.02 gm. of potassium oxalate. A 2.0 per cent solution of potassium oxalate is placed in tubes which are placed in the dry heat sterilizer and dried 10 ml. of blood, immediately after withdrawal, is mixed with the dry powder. The blood was then immediately centrifuged to lessen any spontaneous hemolysis. The supernatant plasma was aseptically removed and placed in a sterile container.

The cultures were grown 18 to 20 hours in a veal infusion broth containing 0.05 per cent dextrose. In performing the test, 0.2 ml. of fresh plasma, not over 24 hours old (since withdrawal), was diluted with 0.8 ml. of 0.85 per cent saline solution in a tube to be used for the test. To this, 1:5 dilution of the plasma, 0.5 ml. of the supernatant fluid of the culture and 0.25 ml. of a 0.25 per cent solution of $CaCl_2$ in 0.85 per cent sodium chloride solution was added and thoroughly mixed. The tubes were then placed in a water bath at 37° C. and the time at which coagulation occurred was noted. Solid coagulation was considered completed when the tube could be inverted without affecting the solid form of the clot, which adheres to the bottom and sides of the

tubes. Usually no fluid, or only small drops escape from the tube. The tubes were left in the water bath for two hours of continuual observation for the complete dissolution of fibrin clot and was regarded positive when the contents of the tube were completely liquified. The test was regarded negative if no dissolution occurred after a period of two hours.

Precipitin Reaction

For the determination of groups of hemolytic streptococci, the original Lancefield method was strictly followed:

I. Immune Sera.

Rabbits were immunized with formalized cultures as follows: The bacterial sediment from 18 hour broth culture was suspended in one-twentieth volume of 0.85 per cent sodium chloride solution to which formalin was added in a final concentration of 0.2 per cent. After 48 hours in the ice box these bacterial suspensions were sterile. Immediately before use they were diluted with physiological salt solution to the original volume of the culture. Daily intravenous injections of 1.0 cc. were given for a week, followed by a week's rest. Two to four series of injections were made. Although good antisera were obtained after two series, four or more courses were sometimes required. On the fifth day after the last injection test bleedings were made and the serum of animals showing a good titer was collected and stored in the ice box without a preservative.

All strains tried gave usable antisera by this method, although some were better antigens than others. After serial subcultures in 10 per cent type specific immune serum, the resulting culture was relatively devoid of type-specific substances and proved the best antigen for inducing the formation of anti-C precipitin specific for each group.

Antisera for the strain of human original (group A) were chiefly those which had been prepared by a method already described employing increasing doses of heat-killed organisms followed by living cultures, although the method already described was satisfactory for these also. With many of the strains of the other groups it was impossible to use a scheme of immunization necessitating the injection of living culture, since too great a loss of animals resulted.

Antisera were tested with extracts of both homologous and heterologous strains of the same group in order to make sure of the presence of the group ant-C precipitin. The type-specific anti-body for a subgroup, or type, was often present in addition to the group anti-C precipitin used in this classification, and was sometimes the only antibody present. Consequently if an extract of the homologous strain were the only one used in testing a serum, a type-specific reaction might be obtained which would mask the group, or anti-C precipitin reaction, and the presence or absence of this anti-C precipitin might not be discovered.

Since this classification is based on the anti-C precipitin reaction, it was essential in testing antisera to employ an extract of a strain of heterologous type but homologous group, as measured by the anti-C reaction.

II. Extracts.

Extracts were made by a method previously employed in preparing the type-specific substance, M, of strains of human origin.

The bacterial sediment from 250 cc. of an 18 hour broth culture was suspended in 5 cc. of physiological salt solution containing sufficient normal hydrochloric acid to make a final concentration of N/20 HCl. The reaction of the suspension was tested with Congo red paper, and if necessary, enough hydrochloric acid was added to turn the paper blue. The tube was then immersed in boiling water for 10 minutes, cooled under running water, and centrifuged. The supernatant fluid was neutralized, the resulting precipitate discarded, and the water-clear supernatant fluid was used in the precipitin test. Obviously, such a crude extract contained a mixture of substances, but these did not interfere with the reaction under consideration.

III. Precipitin Test.

In performing this test, increasing amounts of extract were placed in a series of tubes, usually up to 0.4 cc. with normal salt solution, and a control tube with 0.4 cc. of the same diluent was included. A constant volume of 0.2 cc. of undiluted antiserum was layered in each tube and allowed to stand for 10 to 30 minutes either at room temperature or in the water bath at 37° C. in order to observe ring formation. The tubes were then shaken and incubated for 2 hours at 37° C. in the water bath. Sometimes a larger series of dilutions was employed, but no difference in result was obtained. Extracts from all strains were tested in the same way.

Fermentation Reactions

Gordan (1905a and 1905b) applied fermentation in a broad way, using many disaccharides, monosaccharides and glucosides for the classification of the streptococci.

Many other investigators have been given credit for such work, but a variation of Gordan's method gave satisfactory results. Instead of sugar free beef broth, we used Hartley broth. Hartley broth contains foreign fermentable substances, which were eliminated by the use of controls for each strain of organism. The inoculated control tubes contained no sugars; consequently any reaction shown was due to the foreign sugars. Comparisons were made with the control and "test" tubes. In all cases reactions were found to be clear-cut and definite. The Hartley broth was prepared in 90 ml. amounts with 1% andrades indicator and sterilized at 15 lb for 30 minutes. The various sugars were separately sterilized in eutral concentrated solutions to minimize any hydrolysis. Rare sugars were prepared in 5 per cent solution while others were made up in 10 per cent solutions. They were sterilized in 50 ml. vaccine vials and aseptically capped. For preparing the media, 10 ml. of the concentrated solution was withdrawn with sterile 10 ml. syringe and aseptically injected into 90 ml. of Hartley broth. This broth was then placed in the incubation at 37° C. for 48 hours. If there was no apparent contamination, the media was promptly pipetted aseptically into

tubes in 5 ml. amounts and again incubated to insure sterility.

Heavy 18-20 hour broth suspensions were inoculated into the media and usually a 24 hours incubation was sufficient to bring about differential results, but 5 days of incubation was given to all.

Positive results were those showing definite deeper coloration of the media than the control tubes.

The test involving the use of media containing 4 per cent peptone for the determination of ability of the streptococci to produce ammonia proved early in our experiment confusing and inconsistent. The test was abandoned after several careful tests failed to give consistent results.

Sodium hippurate hydrolysis also seemed frequently to give inconsistent results in spite of meticulous care in its preparation and use of controls. Because of its unpredictable deviations and inconsistency the test was not used.

Table I a

The Basis of the Classification of

the Pyogenic Streptococci

Number of Species	Lancefield Group	Hemolysis	Growth at 10°C	Growth at 45°C	Growth in 6.5% NaCl	Growth in presence of 1.2% M.B.*	Growth in presence of ph 9.6	Strong reduction	Survival 30°	Survival 65°	Active fibrinolysis	Starch Hydrolysis	Esculin Hydrolysis	Milk Curdled	Gelatin liquified	Acid Produced in: Arabinose	Mattose	Sucrose	Lactose	Trehalose	Raffinose	Inulin	Glycerol	Mannitol	Sorbitol	Salicin	Xylose	Species of Streptococcus
10		+	-	-	—	-	-	-	-	-	-	-	±	-	-	-	+	+	+	+	-	-	-	±	-	±		Streptococcus Pyogenes Lancefield A.
14		+	-	-	-	-	-	-	-	-	±	±	±	±	-	-	+	+	±	-	-	-	+	-	-	+		Streptococcus Pyogenes Lancefield C Human "C"
1		+	-	-	—	-	-	-	-	-	-	+	+	-	-	-	±	+	+	+	-	-	-	-	+	+		Streptococcus Pyogenes Lancefield C Animal "C"
1		+	-	-	—	-	-	-	-	-	-	±	±	-	-	-	+	±	+	+	-	-	+	±	±	+		Streptococcus Pyogenes Lancefield Group E
12		+	-	-	-	-	-	-	-	-	±	±	±	±	-	-	+	+	±	±	±	-	±	-	-	+		Streptococcus Pyogenes Lancefield Group G
2		±	-	+	—	-	-	-	-	—	-	-	±	-	-	-	+	+	±	±	+	-	-	-	-	+		Streptococcus Pyogenes Lancefield Group H

* M.B. Methylene Blue

Table I b

The Basis of Classification of

The Viridans and Lactic Streptococcus

Number of Species	Lancefield Group	Hemolysis	Growth at 10° C	Growth at 45° C	Growth in presence of 6.5% Nacl	Growth in presence of 1.% M.B.	Growth in presence of P^h 9.6	Strong reduction	Survival 30 min. 60°	Survival 30 min. 65°	Active fibrinolysis	Starch Hydrolysis	Esculin Hydrolysis	Acid produced in: Milk Curdled	Gelatin Liquified	Arabinose	Maltose	Sucrose	Lactose	Trehalose	Raffinose	Inulin	Glycerol	Mannitol	Sorbitol	Salicin	Xylose	Species of Streptococcus
49		-	-	-	-	-	-	-	-	-	-	-	±	±	-	-	-	+	+	±	±	+	-	-	-	±		Streptococcus Salvarius Viridans
34		-	-	-	-	-	-	-	-	-	-	-	±	±	-	-	+	+	+	±	±	+	-	-	-	±		Streptococcus Salvarius Variation of Viridans *
15		-	-	+	-	-	-	-	-	-	-	±	+	-	-	-	+	±	-	±	±	±	-	-	-	±		Streptococcus Equinus Viridans
4		-	-	+	-	-	-	-	-	-	-	+	+	±	-	±	+	+	+	±	+	±	-	±	±	+		Streptococcus Bovis Viridans
1		-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	±	-	-	-	-	-		Streptococcus Thermophilus Viridans
1		-	+	-	-	-	-	-	-	-	-	+	-	+	-	±	±	+	+	-	-	-	-	±	-	±	±	Streptococcus Lactis Lactic
1		-	+	-	-	+	-	+	±	-	-	-	+	+	-	-	±	±	+	-	-	-	-	-	-	±	-	Streptococcus Cremoris Lactic

* Produces no apparent reaction on blood

Table I.c

The Basis of Classification of

Enterococci Streptococcus

Number of Species	Lancefield Group	Hemolysis	Growth at 10° C	Growth at 45° C	Growth in 6.5% NaCl	Growth in Presence of 1.% M.B.*	Growth in Presence of P^h 9.6	Strong reduction	Survival 60°	Survival 65°	Active fibrinolysis	Starch Hydrolysis	Esculin Hydrolysis	Milk Curdled	Gelatin liquified	Acid Produced in: Arabinose	Maltose	Sucrose	Lactose	Trehalose	Raffinose	Inulin	Glycerol	Mannitol	Borbitol	Salicin	Species of Streptococcus
45		-	+	+	+	+	+	+	+	±	-	-	+	+	-	±	+	±	+	+	±	-	±	+	+	+	Streptococcus fecalis Enterococci
1		-	+	+	+	+	+	+	+	±	-	-	+	+	+	±	+	±	+	+	±	-	±	+	+	+	Streptococcus Liquifaciens Enterococci
15		+	+	+	+	+	+	+	+	±	-	-	+	+	+	±	+	+	+	+	±	-	+	+	+	+	Streptococcus Zymogenes Enterococci
1		-	+	+	+	+	+	-	+	±	-	-	+	+	-	-	+	-	+	±	-	-	-	-	-	±	Streptococcus Durans Enterococci

* M.B. Methylene Blue

IV. Classification of the Streptococcus

In the classification of the streptococcus extreme care and caution must be alertly exercised to obtain uniform and accurate results. In spite of the precautions in technique and interpretations, some groups of the streptococcus were found difficult to classify. The viridans group demonstrated that irregularities and variations very frequently occur. One of its chief variations was its reaction on blood agar. The gamma type of Brown gave reactions identical in every respect to that of viridans type. Those species giving gamma type reactions on blood agar and giving other reactions identical to viridans were classified as variants of the viridans. The most numerous of viridans were the salvarius. Typical salvarius constituted 24.5 per centum of all the species isolated. A typical salvarius giving gamma reaction blood composed 10.7 per cent of all the species. The total salvarius species was approximately 35 per cent. With interest was the discovery that Streptococcus equinus was found in larger number than expected with 7.5 per cent. Several species of streptococcus bovis and thermophilus were found. One strain of streptococcus lactis and cremoris is also included.

The classification of the pyogenic group offered very few obstacles in differentiation. Careful isolation on the blood agar enabled primary differentiation to be established. Great caution must be exercised in the selection of this group for the

so-called alpha-prime of Brown may be classified as a pyogenic group. The Lancefield precipitin test gave unquestionable and dependable results. The other biochemical reactions faithfully correlated with the Lancefield precipitin test. With interest is the pathogenic specie, Lancefield A, which was found and it constituted 5 per cent. Lancefield "C" or the so-called Human "C" which may at times be pathogenic, composed 7 per cent of all the species. Another specie which may be pathogenic, the Lancefield G, was frequently found (6 per cent). Several other species with a sum of 2 per cent were found. The total pyogenic group constituted approximately 20 per cent of the isolated strains. The majority of the pyogenic, viridans, and Lactic species were isolated from the sputum of the nasopharynx.

The species giving the least difficulty in classification were the members of the enterococci. Their reactions were regular and dependable. Streptococcus fecalis was the most abundant of all the enterococci species, 27.5 per cent. The hemolytic enterococci, streptococcus zymogenes was next in order of abundance (7.5 per cent). A few streptocuccus liquifactions and durans were found (1 per cent).

The total number of viridans streptococcus identified in routine classification is 60 per cent of all the strains. The total hemolytic species, 27 per cent, and the remaining per cent is composed of gamma type of Brown, which fell into Salvarius species by biochemical reaction and the lactic species.

The classified and identified species were gathered in a table with skin reactions and eosinophile counts and the unwieldly table was placed in this section for a reference to the subsequent analytical charts and tables.

Species are identified by a serial number which is composed of a number and two letters. Example: 56 - A - S. 56 represents the order number of the specie and the "A" represents the first letter of the patient's name and the "S" represents the source in this case sputum.

TABLE II

Chart of Clinical and Experimental Data Used In This Work

No.	Sex	Age	History	Diagnosis	Eosinophile		Streptococcus	Skin Reactions			
					B.	S.	Species and Source	Vaccine		Filtrate	
								I.	24	I.	24
2205	F.	46	Arthritis		2	D-	Salvarius 1-R-S	-	-	II	-
							Lancefield G 2-R-S	I	?	I	-
							Fecalis 3-R-F	-	-	?	-
16362	M.	58		Bronchial Asthma	2	-	Salvarius 4-H-S	III	-	I	-
							Lancefield H. 5-H-S	-	I	-	-
							Equinus 6-H-S	-	-	II	-
56748	M.	5					Fecalins 7-H-F	?	-	III	-
95480	F.	50	*Eczema *Nostril Trouble		10	-	Salvarius (1) 8-P-S	-	II	I	-
							Lancefield C 9-P-S	-	II	I	-
							Salvarius (2)10-P-S	-	II	II	I
89706	M.	57			5	-	Salvarius 11-S-S	-	II	I	-
							Lancefield A. 12-S-S	-	II	I	-
88478	M.	57		Asthma			Liquifaciens 13-C-F	-	I	I	-
							Zymogenes 14-C-F	-	I	I	-
78387	M.	6	Sneezing Paroxysm		4	-	Fecalis 15-H-F	-	-	?	?
76021	M.	58	Urticaria Dyspnea Wheezing		6	-	Salvarius 16-P-S	-	-	?	?
							Lancefield A 17-P-S	-	II	?	?
							Salvarius 18-P-S	-	I	I	?
							Zymogenes 19-P-F	-	-	?	-
111284	M.	44	Hayfever Asthma		8	-	Thermophilus 20-A-S	-	?	I	-
							Lancefield A. 21-A-S	-	?	I	-
							Salvarius 22-A-S	-	?	I	-
							Fecalis 23-A-F	-	?	I	-
111739	M.	11	Asthma		16	-	Fecalis 24-S-F	-	-	-	-
111987	M.	58	Asthma		3	-	Fecalis 25-C-F	I	?	IIII	-
							Zymogenes 26-C-F	?	?	IIII	-
112855	F.	3			7	-	Fecalis 27-C-F	-	-	I	-
97700	M.	74	Ch. Strep. Bron	Allergic Rhinitis	1	-	Salvarius 28-S-S	-	IIII	II	?
							Lancefield C 29-J-S	-	III	II	?
							Salvarius 30-J-S	-	III	III	?
77698	M.	71	Asthma		1	-	Fecalis 31-C-F	-	?	I	-
70990	M.	61			3	-	Salvarius 32-F-S	-	-	I	-
							Lancefield G.33-F-S	-	I	I	?
							Equinus 34-F-S	-	I	I	-

67866	F.	75			X	X	Salvarius	55-H-S	-	-	II	I
							Lancefield C	56-H-S	-	I	II	I
							Salvarius	57-S-S	-	I	III	-
55907	F	62	Urticaria Bronchitis		X	X	Fecalis	58-H-S	-	?	I	-
							Zymogenes	59-H-S	-	?	I	-
24737	M	72		Bronchial Asthma	4	-	Salvarius	40-T-S	-	I	III	-
							Lancefield C	41-T-S	-	I	II	-
							Equinus	42-T-S	-	?	II	-
							Fecalis	43-T-F	-	-	I	-
15246	M	57			1	-	Salvarius	44-H-S	-	II	II	-
							Lancefield C	45-H-S	-	-	I	-
							Salvarius	46-H-S	I	?	II	-
112859	F	48	Arthritis		1	-	Fecalis	47-C-F	-	-	I	-
99306	M	28	Vasomotor Rhinitis		1	-	Salvarius	48-D-S	I	II	I	IIII
							Lancefield A	49-D-S	-	I	?	IIII
108476	F.	52 42	Asthma		1	1	Salvarius	50-H-S	-	I	II	-
							Lancefield C	51-H-S	-	-	I	-
							Salvarius	52-H-S	-	?	I	-
108522	M	42		Asthma Allergic Rhinitis	8	12	Fecalis	53-H-F	-	-	I	-
							Zymogenes	54-H-F	-	-	II	-
108870	M.	61			5	-	Salvarius	55-C-S	?	-	IIII	?
							Lancefield C	56-C-S	?	-	?	-
							Salvarius	57-C-S	-	-	II	-
48661	M.	65		Bronchial Asthma	10	-	Salvarius	58-P-S	I	III	II	-
							Equinus	59-P-S	I	II	III	-
63388	M.	21			1	-	Salvarius	60-G-S	-	?	?	-
							Lancefield G	61-G-S	-	I	?	-
							Equinus	62-G-S	-	II	?	-
90282	M.	6	Sneezing Nasal Obst.		5	-3	Salvarius	63-P-S	-	?	-	?
							Lancefield C	64-P-S	-	I	II	I
							Equinus	65-P-S	-	I	I	?
31103	M.	9				25	Salvarius strain	(1)66-C-S	-	?	I	-

							Salvarius strain(2)67-C-S	-	?	?	-
95051	F.	24			2	-	Salvarius 1	68-W-S	-	-	I	?
							Lancefeild C	69-W-S	-	-	I	-
							Salvarius 2	70-W-S	-	III	I	1
70781	F	27	*nasal obs. dyspnea and wheezing		7	-	Salvarius	71-C-S	?	?	III	-
							Lancefield C.	72-C-S	-	?	III	-
70434	M.	25	Attacks wheezing and dyspnea		-	11	Salvarius	73-F-S	-	I	I	-
							Lancefield C	74-F-S	I	I	II	?
113698	F.	40	*Asthma Urticaria Eczema		1	-	Equinus (1)	75-F-S	-	-	I	-
							Salvarius (2)	76-F-S	-	?	II	-
115869	M.	60	*Asthma Eczema Hayfever	Asthma Bacterial	3	-	Salvarius (1)	76-I-S	II	-	?	-
							Salvarius (2)	77-I-S	III	-	I	I
115137	M.	8			15	-	Bovis	78-J-S	-	?	I	-
							Lancefield C.	79-J-S	-	I	I	-
							Salvarius	80-J-S	-	I	II	?
114066	F.	55	Nonseasonal Asthma Eczema	Bacterial Asthma	2	9	Salvarius	81-E-S	I	-	-	-
							Equinus	82-E-S.	I	-	II	-
							Fecalis	83-E-F	I	-	I	-
36977	M.	10	Bronchial Asthma		22	6	Salvarius	84-R-S	-	I	II	I
							Lancefield E	85-R-S	-	II	II	III
							Fecalis	86-R-F	-	-	I	—
6794	F.	35	Bronchial	* * Asthma	8	-	Fecalis	87-U-F	-	III	II	-
52036	M.	59	Cough with dyspnea		1	-	Salvarius (g)	88-S-S	-	-	I	-
53191	M	66	Bronchial	Asthma	13	10	Bovis	89-C-S	?	III	II	-
							Equinus (g)	90-C-S	-	I	II	I
68042	M.	53	*Asthma Hayfever	Bacterial Asthma	1	10	Salvarius	91-L-S	-	-	II	?
							Fecalis	92-L-F	-	-	I	-
70860	F.	30		Bacterial Asthma	4	-	Equinus	93-Q-S	-	?	I	-

89084	F.	46	*Asthma Dyspnea Cough	Bacterial Asthma	–	60	Salvarius	95-N-S	–	II	III	III
							Salvarius (2)	96-N-S	?	III	?	II
							Fecalis	97-N-F	–	II	I	I
							Zymogenes	98-N-F	–	III	?	–
95589	M.	8	*Asthma		7	–	Fecalis	99-H-F	?	I	II	–
110464	M.	55		Bronchial Asthma	4	–	Fecalis	100-B-F	–	–	?	?
2221	F.	57	Non-seasonal Wheezing Dyspnea		3	–	Salvarius I	101-C-S	–	I	3	–
							Lancefield C.	102-C-S	–	–	I	–
							Salvarius 2	103-C-S	–	?	?	–
14223	F.	19		Allergic Asthma	6	10	Salvarius I	104-G-S	–	I	I	I
							Lancefield A.	105-G-S	?	–	I	I
5937	M.	44		Bronchial Asthma Hayfever	1	–	Fecalis	106-M-F	–	–	4	–
16979	M.	56	Sneezing Wheezing		3	10	Salvarius	107-H-F	–	–	2	I
95599	F.	6	Hayfever Urticaria		2	–	Fecalis	108-A-F	–	I	2	–
97190** skin	F.	26	Urticaria Occipital Headache		3	–	Lancefield G. skin	109-B-E	–	2	?	–
72168	M.	63	Urticaria Dyspnea		–	30	Salvarius	110-M-S	–	–	–	–
							Lancefield A.	111-M-S	–	–	2	–
							Equinus	112-M-S	3	?	2	–
58767	M.	49		Vasomotor Rhinitis	5	–	Fecalis	113-A	–	–	2	?
58742	M.	50	Dyspnea Urticaria		–	–	Salvarius	114-G-S	–	–	I	–
							Lancefield G	115-G-S	–	2	I	–
							Salvarius 2	116-G-S	–	I	–	–
117007	M.	5	Non-seasonal Nasal obstruction		–	–	Bovis	117-F-S	–	?	I	–
							Equinus	118-F-S	–	?	I	–
113465	F.	17	*Difficulty in breathing *Allergic Rhinitis		4	–	Salvarius	119-C-S	–	I	I	–
							Fecalis	120-C-F	–	–	I	–
102592	F.	45		Asthma		10	Salvarius	121-G-S	?	II	I	I

				*Allergic Rhinitis			Equinus	122-C-S	?	III	?	-
81730	F.	58		Bronchial Asthma	2	20	Salvarius	123-H-S	-	?	?	III
							Lancefield C	124-H-S	-	-	?	III
81161	F.				1		Equinus2)	125-B-S	-	I	I	-
							Lancefield H	126-B-S	-	II	I	?
							Salvarius (1)	127-B-S	-	I	I	?
108594	F.	46	*Eczema Wheezing Dyspnea		1	-	Salvarius	128-C-S	-	-	III	-
							Lancefield animal pyogenes	129-C-S	-	-	III	-
							Fecalis	130-C-F	-	-	III	-
113207	M.	65	Non-seasonal sneezing with coryza		3	-	Fecalis*	131-S-S	—	-	III	-
							*Broth-II					
115604	F.	42	Eczema Food Sen. (egg)		9	60	Salvarius (2)	132-B-S	1	-	II	-
52560	M.	43	*Chronic bronchitis Eczema		-	-	Bovis	133-F-S	?	I	II	-
0813	F.	5	Non-seasonal wheezing and dyspnea		2	1	Fecalis	134-A-F	-	II	II	-
102455	M			Bacterial Asthma		80	Salvarius	135-M-S	-	-	I	-
21354	M	58	Asthma Allergy Anophylaxis	" "	2	-	Fecalis	136-M.F	++	-	+	-
21354	M	58		Bronchial Athma	2	-	Salvarius	137-M-S	-	-	I	-
							Lancefield s.C.	138-M-S	?	-	?	-
							Salvarius (?)	139-M-S	-	-	?	-
							Fecalis	140-M-F	-	I	?	?
							Durans	141-M-F	-	?	?	-
113027	F.	60	Asthma Hayfever				Fecalis	142-S-F	-	-	?	-
							Fecalis (g)	143-S-F	-	-	?	-
115939	M.	50					Fecalis	144-L-F	-	-	I	-

10385	M.	34			5		Zymogenes	145-L-F	-	-	2	-
							Salvarius	146-F-S	-	-	2	-
							Lancefield G.	147-F-S	-	-	I	-
							Cremoris	148-F-S	-	-	2	-
							Fecalis	149-F-F	-	I	?	?
							Zymogenes	150-F-F	-	I	I	-
85385	M.	49	Bronchial Asthma		L	-	Salvarius (1)	151-S-S	-	-	3	2
							Lancefield C.	152-S-S	-	2	2	-
							Salvarius (2)	153-S-S	-	2	2	-
18080	M.	16	Sneezing nasal discharge itching eyes		3		Salvarius (1)	154-M-S	I	I	?	-
							Lancefield A.	155-M-S	-	I	?	-
							Salvarius (2)	156-M-S	?	-	I	-
55907	F.	62	Bronchitis		4	-	Fecalis	157-M-F	-	?	I	-
							Zymogenes	158-M-F	-	?	I	-
113208	M.	47	Bronchial Asthma		2		Salvarius (I)	159-D-S	-	I	I	-
							Lancefield A	160-D-S	-	-	I	-
							Salvarius (2)	161-D-S	-	-	I	-
117612	F.	25	Non-seasonal Asthma				Equinus	162-S-S	-	-	I	-
							Salvarius	163-M-S	-	I	I	-
81445	M.	51	Skin case Eczema Urticaria		8		Fecalis	164-M-F	-	?	I	-
							Zymogenes	165-M-F	-	?	I	-
17389	F.	38	Skin cse. rash beats			-	Fecalis	166-M-F	-	-	?	-
116498	M.	26	Asthma no hayfever		2	-	Salvarius	167-V-S	-	-	I	-
							Lancefield C.	168-V-S	-	?	?	-
							Fecalis	169-V-F	-	-	2	-
114416	M	47	Broch. Asthma		2		Salvarius	170-E-S	I	I	4	-
							Durans	171-E-F	I	I	3	-
							Salvarius (2)	172-E-S	-	?	3	-
4394	F	54	Hayfever Eczema		6	-	Fecalis	173-C-F	2	-	2	-
							Zymogenes	173-C-F	I	I	4	-
19505	F.	33	Puritis				Zymogenes	174-L-E	?	I	2	-
117120	F.		ani				Salvarius (1)	175-C-S	-	I	I	-
			Impairied				Salvarius (2)	176-C-S	-	?	2	-
			Breathing sneezing				Equinus	177-C-S	-	I	I	-
1833	F.	33	Nasal Disch.		8	-	Fecalis	178-M-F	-	I	I	-

87578	M.	62	Drainage Asthma Emphysema		4	-	Fecalis	179-P-F	-	I	I	-
							Zymogenes	180-P-F	-	I	I	-
116567	M.	52		Asthma	7	-	Fecalis	181-S-F	-	?	I	-
							Zymogenes	182-S-F	-	?	I	-
Brown, A	M			Asthma			Fecalis	183-B-F	-	-	I	-
114817	F			Asthma		-	Salvarius	184-H-S	-	?	2	-
							Lancefield C.	185-H-S	I	-	I	-
							Lactis	186-H-S	-	2	3	I
							Fecalis	187-H-F	I	-	I	-
103183	F			Bronchiatisis			Salvarius	188-A-S	-	-	2	-
							Lancefield G.	189-A-S	-	-	2	-
100562	M.			Asthma			Salvarius (1)	190-M-S	?	-	2	-
							Lancefield G.	191-M-S	?	-	3	-
							Salvarius (2)	192-M-S	?	I	4	-
							Fecalis	193-M-F	I	I	3	-
113704	F						Salvaris (I)	194-K-S	2	I	I	-
							Lancefield A.	195-K-S	?	I	?	I
							Salvarius (2)	196-K-S	3	-	3	-
							Fecalis	197-K-F	?	3	I	-
							Zymogenes	198-K-F	I	3	2	?
113704	F						Salvarius	199-K-S	2	I	I	-
							Lancefield A.	200-S	?	I	I	-

*Sp - sputum

V. Skin Tests.

To avoid discrepancies in the correlation of the skin reactions to the species of streptococcus, it is necessary to be aware of possible sources of error which would result in confusion and inaccuracy of results.

The first source of error would be the skin reactions. Since the skin reactions are read routinely in large numbers, the error would not be significant in the actual reading. The age of the patient may have direct influence on the skin reactivity; as the age increases there may possibly be increased predisposition of the patient for bacterial infection due to the lowered resistence. Although skin reactions are fairly well established, a manifestation of specific reaction, it is still worthy to never neglect the possibility of the presence of non-specific reaction due to age, media, or other non-specific bacterial substances. The necessity of controls and meticulous care in testing cannot be over-stressed.

The effect of age upon the skin reaction. In table III age groups are arranged in decades, and the percentum of the strains from the members of this age group are tabulated so that the reactions correspond with that particular age group. The percentum of positive reacting strains roughly seems to indicate an increased reactivity as the age advances. The delayed reaction of the vaccine which is generally accepted as indicative of bacterial allergy gradually increases with age. While the delayed reaction to the filtrate in the tabulation decreases with age, whatever the effect age may have upon the skin reactivity, it cannot be found without use of normal non-allergic controls for comparison. Since the cases are all of the same nature, the age factor may eliminate itself. It is necessary, however, to keep such factors in mind as possible sources of discrepancy and misinterpretation.

Effect of age upon the eosinophile count. We have considered the age factor in another phase. A high eosinophile count, thought to be indicative of allergic condition, has been treated in the same manner as the skin reaction. Although eosinophilia is a non-specific phenomena, many investigators support its diagnostic value. Should skin reactivity increase with advancing age, it should be possible to observe increase in eosinophile counts. The sputum eosinophile counts seem to increase slightly with age. The age group 1-10 has 2.5 per cent eosinophiles in the sputum. The per centage increases gradually with age. At the age group 41-50 the percentage is 8.7 and gradually decreases to 5.7 per cent

Table III.

Relations of age groups and skin reactions larger than 1 cm.

Age groups in decades	Vaccine			Filtrate		
	Per cent of strains giving:			Per cent of strains giving:		
	I.R.	D.R.	N.R.	I.R.	D.R.	N.R.
1-10	none	41	59	70	none	30
11-20	12	49	39	62	25	13
21-30	none	35	65	47	5	48
31-40	7	50	43	78	14	8
41-50	12	37	51	87	9	4
51-60	21	35	44	83	8	9
61-70	15	45	40	95	none	5
71-80	none	63	37	100	none	none

at 6-70 years. The blood eosinophile at 1-10 years is 4 per cent; gradually rises to a peak at 11-20 with a count of 6.5 per cent. Then again it gradually declines to 2.7 per cent at the age group of 21-30 years. From 21-30 to 61-70 there is gradual rise to 6 per cent. Just how much significance these eosinophile changes bear in relation to age, cannot be stated. So we must conclude until further experimental data is available that advancing age apparently affects the eosinophile count and also that advancing age affects the type of skin reaction elicited. In this experiment the age factor will not be corrected, although it may seem desirable to do so.

Table IV

Average eosinophile counts in 90 patients
According to ages in decades

Age Groups tested	Number of cases	Average percent of eosinophiles	
		Blood	Sputum
I-I0	I0.0	4.0	2.5
II-20	4.0	6.5	2.5
2I-30	8.0	2.7	2.6
3I-40	7.0	2.5	0.0
4I-50	I5.0	3.0	0.7
5I-60	17.0	3.5	3.6
6I-70	7.0	6.0	5.7
7I-80	4.0	I.5	0.0
Eosinophile average for all ages.		3.8	3.3

VI. A Correlation Of Experimental Results.

The different types of reactions to the vaccine and filtrate. In skin test reactions there is exhibited an interesting phenomena in the behavior of the vaccine and the filtrate. It has been consistently observed that the largest percentum of vaccine reactions are delayed; while largest filtrate reactions are immediate. The immediate vaccine reaction may be due to the small amount of culture media injected with the vaccine. However, cases are known in which immediate reaction responded favorably to specific desensitization which indicates that the reaction is due to specific bacterial substance and not to the media.

The delayed 24 hour reaction is thought to indicate bacterial allergy. The large percentage of immediate reactions to the filtrate substance may be due to the presence of peptone in the broth media and other possible substances. The delayed filtrate reaction is, however, only relative, for the percentage of the vaccine delayed reaction is much greater. The skin reactions for 200 strains were tabulated in such a manner that the relative values for the vaccine and the filtrates could be compared. Table V reveals that 3.5 percentum of the strains gave an immediate reaction to the vaccine and that 9.75 percentum gave delayed reaction; in contrast, 19 per centum of the strains gave immediate reactions to the filtrate and 3.25 percentum to the delayed reaction. The large percentum of the strains reacting immediately to the bac-

Table V

Percentage of 200 strains of streptococci which gave various types of skin reactions in the patients from whom isolated.

size of Reaction	Vaccine			Filtrate		
	Immediate Reactions	Delayed Reactions	Total	Immediate Reactions	Delayed Reactions	Total
No Reactions	21	15	36	4.3	22.5	26.5
I. cm.	2	6	8	10.	2	12
2. cm.	I	2.5	3.5	5	.4	9
3. cm.	.5	I	I.5	3	.5	3.5
4. cm.	0.	.25	.25	I.	.25	1.25
Total percentage of types of reaction.	3.5	9.75	13.25	19.	5.25	28.

terial filtrate may be due to the soluble bacterial exotoxin in the culture filtrate. The presence of peptone and other possible foreign irritants may be another possible factor. The delayed filtrate reaction would possibly be comparable to the delayed vaccine reaction if the filtrates contained equivalent amounts of bacterial substances as found in the vaccine. An interesting check could be performed by injecting the unwashed bacteria, its soluble products, and the culture media. It may be safe to anticipate that a result analogous to the sums of the percentum of the vaccine and filtrate immediate reaction, and vaccine and filtrate delayed would be present. Immediate reactions to unwashed whole bacteria and media may be 22 percentum on vaccine and 13 percentum on filtrate delayed reaction. However, such results must be experimentally and clinically confirmed or disproved.

We took this digression for the purpose of establishing some definite basis to be accepted when interpreting the skin reactions. The significance of vaccine and filtrate reactions are yet to be experimentally supported. Our purpose is to enlighten somehow the pathway toward the explanation of the true relationships of these substances and a correlation of the species of streptococcus with the various types of skin reactions.

It occurred to us that certain types or certain patterns of skin reactions may be due to some species or groups of streptococci.

A correlation of species of pyogenic group with various types of skin reactions. Table VI tabulates the various skin reaction behaviors of the species in the pyogenic group. The table reveals that the majority of the members of any one specie gives delayed vaccine and immediate filtrate reactions. The percentage of the total reacting hemolytic species giving delayed reaction is 21 per cent, while the percentage of species giving immediate reaction is 23 percent. The group as a whole gave 5 percent immediate reaction to the vaccine and 4.25 percent to filtrate delayed reaction. As a group the hemolytic species does not show any outstanding characteristics. It is interesting to note that a direct correlation exists between table VI and V, and also that the streptococcus pyogenics of Lancefield A was the only member of the pyogenic group giving an immediate reaction to the vaccine. Moreover, it does not give any delayed skin reaction to the vaccine. Its reaction to the filtrate is characteristic of Lancefield A species. We have shown in table V that the majority of the strains gave large immediate reaction but only a negligible delayed filtrate reaction. On the contrary, the skin reactions of Lancefield A species give delayed filtrate reactions equivalent to its immediate reactions. The remaining species show no marked deviation from the other members of the group.

The types of skin reaction given by the Lancefield A may be due to several factors. The pathogenic nature of the organ-

Table VI

Percentage of various types of skin reactions of the pyogenic streptococcus larger than 2 cm.

Species of streptococcus	Vaccine		Filtrate	
	I.R.	D.R.	I.R.	D.R.
Streptococcus pyogenes Lancefield A.	5	None	2.5	2.5
Streptococcus pyogenes Human "C"	None	5.	9.	1.5
Streptococcus pyogenes Animal "C"	None	None	.25	None
Streptococcus pyogenes Lancefield E.	None	None	.25	.25
Streptococcus pyogenes Lancefield G.	None	4.	10.	None
Streptococcus pyogenes Lancefield H	None	12.	None	None
Total reacting hemolytic species.	5	21.	23.	4.25

ism may contribute certain factors. Soluble exotoxins as erythrogenic toxins may be responsible. Perhaps the antigenic make-up of the species can explain the behavior.

This peculiarity would be of value in diagnosis and treatment if firmly established and confirmed by other investigators.

Other species of the pyogenic group do not exhibit such a peculiarity; so that from the experimental data, it is possible to say that the Lancefield A gives a skin reaction characteristic and distinct from the other members of the group. Before any definite statement can be made, further experimental investigation is necessary for evaluation of the behavior..

A correlation of the species of viridans streptococcus with skin reactions. Table VII gives the percentum of various types of skin reactions due to the viridans and lactic streptococcus.

The salvarius of the viridans and the gamma varieties exhibit similar types of skin reactions. The percentum of salvarius of viridans variety giving immediate reactions to the vaccine is 2, while salvarius of gamma variety give 1.5. On delayed vaccine reaction viridans salvarius gave 4.5 percentum and gamma salvarius, 4. A close similarity exists in the filtrate reactions also.

Equinus also exhibits skin reactions very similar to salvarius species. The streptococcus bovis, however, seems to give a higher percentum of immediate and delayed vaccine reaction than

Table VII

Percentage of various types of skin reactions of the viridans streptococcus larger than 2 cm.

Species of streptococcus	Vaccine		Filtrate	
	I.R.	D.R.	I.R.	D.R.
streptococcus salvarius viridans.	2.	4.5	10.	2.
Streptococcus Salvarius "Gamma"	1.5	4.	9.5	.7
Streptococcus Equinus Viridans.	1.6	5.	11.6	None
Streptococcus Bovis Viridans.	6.	6.	None	6.
Streptococcus Thermophilus Viridans.	None	None	None	None
Streptococcus Lactis Gamma.	25.	None	25.	None
Streptococcus Cremoris Gamma.	None	None	25.	None
Total reacting viridans	11.	21.	31.	8.7
Total reacting Lactic	25.	None	50.	None

other members of the group. Also it was responsible for the highest percentum of delayed skin reactions with the filtrate. The filtrate preparation of streptococcus bovis did not give any immediate skin reactions. It gave the highest percentum of immediate reaction of the groups. This distinction may also be of diagnostic value, but as yet it is difficult to evaluate the significance of this behavior of the species.

Due to the fact that only one strain of lactis and cremoris were studied, it is hardly practical to discuss them much. But it was evident that they seemed to give skin reactions of a different type or pattern than the members of the other groups.

A correlation of species of enterococci with the type of skin reactions. Table VIII is a tabulation of the skin reactions for the species of the enterococcus group. In general, the reactions are not drastically different from the reactions obtained from other groups. However, there are no delayed reactions to the filtrate.

The largest percentum of the skin reactions given by members of the group is a delayed reaction to the vaccine and an immediate reaction to the filtrate. This type of reaction may be due to the fact that the filtrate of the enterococcus group does not contain soluble bacterial substances which are responsible for delayed filtrate skin reaction. In the case of the enterococcus group, the bacterial substances which are responsible for the de-

Table VIII

Percentage of various types of skin reactions of the Enterococci streptococcus larger than 2 cm.

Species of Streptococcus	Vaccine		Filtrate	
	I.R.	D.R.	I.R.	D.R.
Streptococcus Fecalis Enterococci	1.2	2.2	9.5	None
Streptococcus Liquifaciens Enterococci	None	None	None	None
Streptococcus Zymogenes Enterococci	None	3.6	10.	None
Streptococcus Durans Enterococci.	None	None	25.	None
Total Reacting Enterococci	1.2	5.8	44.5	None

layed skin reactions seem to be found only in the vaccine or the bacterial cell. Without further experimental support we cannot definitely be justified in stating its significance.

To our investigations of the various species of each group of streptococci we find there are species which give a definite type of skin reaction. From the experimental data obtained it is possible to state that the species are responsible for certain types of skin reactions. At present the responsible factors of the skin reactions characteristic of certain species cannot be definitely stated. In the case of the Lancefield A we have mentioned the possible significance od erythrogenic toxins. In the case of the enterococcus group where no delayed reaction is given by the filtrate, we had suggested the possible nature of the bacterial cell or lack of soluble bacterial subetances in the filtrate to give specific delayed reaction.

A correlation of the groups of streptococci with the skin reactions. Probably the groups of the streptococci may have a certain influence on the types of skin reactions obtained from the vaccine and filtrate preparations. In table IX we have tabulated percentum of various types of skin reactions given by different groups of streptococci. We note that the pyogenic and the viridans groups gave, in general, similar types or patterns of reactions. The viridans group gave a higher percentum of immediate reactions to the vaccine than any one group. Also the viridans

Table IX

Percentage of various types of skin reactions larger than 2 cm. due to different groups of streptococcus.

Groups of Streptococcus	Vaccine		Filtrate	
	I.R.	D.R.	I.R.	D.R.
Pyogenic Streptococcus	5	21	25	4.25
Viridans Streptococcus	11	21	31	8.7
Lactic Streptococcus*	25	None	50	None
Enterococci Streptococcus	1.2	5.8	44.5	None

* 2 strains

group gave the highest percentum of delayed reactions to the filtrate.

Since the lactic group was only composed of 2 strains, we will not accept its reactions as being very significant.

The enterococcus group gave a curious type of skin reaction not found in the other groups. The filtrates of the enterococci do not produce any delayed reactions and this seems to be very characteristic of the enterococcus group. The percentum of the strains giving delayed reactions to the vaccine seems to be much lower than those of other groups, i.e., pyogenic or viridans groups.

The mechanism of the various types of reactions is difficult to determine. However, many probable reasons for its behavior can be offered, but, since at present we are not concerned with the mechanism of the reactions, little discussion has been made regarding this.

A correlation of high eosinophile counts and the types of skin reaction. The purpose of this experiment was to determine if certain species or groups of streptococcus were responsible for any one type or pattern of skin reactions.

Several investigators have attributed the high eosinophile counts as being significant in allergic conditions when parasitic manifestations have been excluded. Since high eosinophile counts have been used as a diagnostic aid, we felt that possibly there may be a correlation between the high eosinophile counts and the

Table X

A correlation of a high eosinophiles count

and the types of skin reactions.

Types of skin reaction	Eosinophile percentage		total
	Blood	Sputum	
Vaccine positive and Filtrate positive	11	9	20
Vaccine positive and Filtrate negative	9	0	9
Vaccine negative and Filtrate positive	5	23	28
Vaccine Negative and Filtrate Negative	8	0	8

types of skin reactions, for both are thought to be manifestations of allergic conditions.

In table X an attempt is made to correlate high eosinophile counts with various types of skin reactions. Those patients having very high (11 percent) blood eosinophile counts gave positive vaccine and positive filtrate types of reactions. The sputum eosinophile counts of those patients with high blood eosinophile counts also were high (9 percent). Patients having high (23 percent) sputum eosinophile counts gave vaccine negative and filtrate positive types of reactions. Patients having no sputum eosinophile gave positive vaccine and filtrate-negative or vaccine - negative and filtrate - negative types of reactions.

Individuals having high eosinophile counts of the sputum give predominence of certain types of skin reactions. A high blood eosinophile count does not seem to have any influence upon the presence of eosinophiles in the sputum.

VII. DISCUSSION.

1) A careful primary isolation on blood agar was most valuable in grouping the streptococcus. To obtain uniform and consistent results in the classification of the streptococcus, extreme care and caution had to be exercised constantly. The various biochemical and tolerance tests faithfully differentiated most of the species. We agree with Sherman (1938) that the viridans group occasionally yields inconsistency in classification in spite of the precautions in technique and interpretations. One of the most important and conclusive tests was the Lancefield precipitin test for differentiating the species of the pyogenic group.

2) We agree with Hooker and Anderson (1928) that the alpha type of Brown predominates in allergic patients. We have found that 20 percent of the strains belonged to the pyogenic group, but cannot agree with Walker and Atkinson (1919) that the pyogenic strains predominate in allergic individuals.

3) Of the stool specimen of these patients, 27.5% yielded streptococcus fecalis which is known to be the predominant organism of the normal intestinal tract.

The presence of Lancefield A, pyogenic streptococcus is interesting because of its potential pathogenic property.

Streptococcus salvarius of the viridans group formed a high percentage of the strains isolated. The significance of

streptococcus salvarius is difficult to evaluate as an etiological agent in allergic conditions.

4) The necessity of careful reading and interpretation of the skin reactions is stressed. We believe with Benson (1932) that false positive skin reactions are frequently interpreted as a specific reaction and that carefully arranged controls are necessary to eliminate the non-specific reactions.

5) The age as a possible factor in altering the skin reactions seems to have no appreciable significance, but we are, however, inclined to agree with Brown (1925) that skin reactivity increases with age.

6) The significance of the types of skin reactions to vaccine and filtrate may be explained by the investigations of W. T. Vaughan (1931), and Thomas and Tounrt (1932) who claimed that delayed, not immediate skin reactions are indicative of bacterial allergy. Although in this experiment we have found a large percentage of the strains giving delayed reactions to vaccine, we cannot satisfactorily explain the significance of the delayed skin reactions.

7) Since group A of Lancefield gives a definite and a characteristic type of skin reaction, we are inclined to believe that specied specificity cannot wholly be disregarded. The types of skin reactions given by the group A may be due to the specific nature of the species. If so, the characteristic skin reactivity would have a definite value in diagnosis.

8) The viridans and the gamma variety of streptococcus salvarius gives the same type of skin reaction, however. Streptococcus bovis of the same group exhibits a distinctly characteristic type of skin reaction.

9) All members of the enterococcus group give similar skin reactions.

10) There are similarities between the types of skin reactions given by the pyogenic and the viridans groups, while the enterococcus group differs in its behavior by eliciting no delayed filtrate reaction.

11) Patients having high eosinophile counts gave a high incidence of certain types of skin reactions. From the experimental data this behavior may seem significant, but further investigation is desirable.

VIII. SUMMARY:

A chronological review and study of the literature pertaining to anaphylaxis and allergy has been included to strengthen the probable significance of the anaphylactic mechanism of altered reactivity in bacterial allergy.

The role of bacteria and the probable mechanism of bacterial allergy also have been chronologically reviewed and discussed.

A review of the literature pertaining to the significance of species of streptococci in allergic conditions, especially of the upper respiratory tracts and its correlation with types of skin test reactions reveals that the viridans group of streptococci, and to some extent the pyogenic group, have been quantitatively studied without regard to possible speciesd specificity and types of skin reactions.

The relative significance of immediate and delayed vaccine reactions in bacterial allergy was determined by a study of skin reactions to 200 strains. The study revealed predominence of delayed vaccine reactions and immediate filtrate reactions.

A qualitative and quantitative effect of age upon the skin test reactivity reveals a slight increase of reactivity with age.

When the species used in autogenous vaccines were classified and correlated with skin reactions, a consistent relationship is observed in all groups of streptococcus, except the Lancefield A group of the pyogenic group and streptococcus bovis

of the viridans group. This suggests that these species give characteristic reactions which possibly are of diagnostic value.

A comparison of the skin test reactions of the members of salvarius and enterococcus groups reveals no outstanding relationship between the types of skin reaction and the species of streptococcus.

Aside from these peculiar behaviors of the group A and streptococcus bovis, the relationship of species to the types of skin test reactions reveals lack of correlation.

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