Chemo-immunological Studies on Pneumococcus.

Report of Dr. Avery with Drs. Tillett, Julianelle, Goebel, Dubos and Dawson.

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X. Publications.

I. Antigenic Dissociation.

In the last report the theory of antigenic dissociation and its significance in Pneumococcus immunity was discussed in detail. During the past year new facts in support of this theory have been acquired and the principles involved have been experimentally applied in the study of active and passive immunity. In order to establish the sequence of thought between the work previously recorded and that to be discussed in the present report, the results of recent chemo-immunological studies may be briefly summarized as follows: Chemically the bacterial cell is composed, among other things, of two constituents each belonging to two wholly different classes of substances and both forming together the major part of the cell as a whole. These two components, - one carbohydrate and the other protein - constitute a unique system which determines both the type-specificity and the antigenicity of the cell. The protein is the somatic substance racially common to all pneumococci, while the carbohydrate, the capsular material, is chemically different and serologically specific for each type. In this system the protein is constantly present as an essential constituent of the cell protoplasm; the carbohydrate, on the other hand, is the synthetic product of a specialized function which is independent of the purely vegetative processes of cell reproduction and growth. By reason of the chemical nature of the substance elaborated, this function bestows upon the cell highly differential properties which biologically endow it with type-specificity and morphologically distinguish it with a well defined capsule. This special function is inhibited or suppressed whenever the cell is subjected to an untoward cultural environment, and is enhanced whenever

the organism is grown in the animal body. The presence or absence of carbohydrates determines the immunological specificity of the cell and modifies its antigenic nature; for, pneumococci which by chemical or biological methods have suffered loss of this substance no longer exhibit the specific reactions of the encapsulated forms from which they were originally derived. In more recent studies on bacterial variation, to be discussed later in this report, the appearance of variants in a given culture is shown to be intimately, if not casually, related to the loss of the physiological function of elaborating this specific substance.

Apart from the importance of the carbohydrate as the determinative substance of type specificity, this constituent is unique in that when chemically isolated in protein-free form, it still retains the capacity to react with antibodies induced by immunization with the whole cell but loses completely the power to stimulate these same antibodies when injected by itself into the animal body. The polysaccharides of pneumococcus, therefore, belong to the group of immunologically important substances which Landsteiner has called haptens. In cellular union these substances constitute the type-specific antigen, separately they are devoid of antigenic function. Evidently, their antigenicity is dependent upon the presence in the cell of some other substance from which they are easily dissociated in the form of haptens. Upon conditions determining the physical or chemical stability of this combination rests the antigenic effectiveness of any given type of pneumococcus, and conversely the more easily dissociable this complex is, the less efficient it is as antigen; that is, the antigenic potency of any given type of pneumococcus is

inversely proportional to the rate and extent of dissociation of the haptaphore group.

This, in brief, is the essence of the theory of antigenic dissociation. Its significance in the problems of antipneumococcus immunity at once becomes clear when it is realized that the only specific therapy of the disease at present available is dependent upon the use of type-specific serum, the potency of which is in turn directly related to the antigenic quality of the organisms used in its production. As pointed out in the last report the titre of antipneumococcus serum in terms of type specific antibodies is conditioned by a balance between those factors of the animal body which bring about antigen cleavage and those chemical properties of the bacterial cell which determine the stability of the specific antigenic complex of each type.

The factors relating to the animal body will be discussed in the experimental work on the "Natural resistance and acquired immunity". The relation between chemical constitution and antigenic stability is being studied from two viewpoints: - 1) Selective fixation of antigen by chemical agents, and 2) Chemical changes occurring during autolysis.

1) Selective fixation. Assuming that the union between pneumococcus hapten (polysaccharide) and its antigenic activator is a labile one, a search is being made for chemical reagents which might "fix" this linkage and by stabilizing the compound prevent or inhibit subsequent dissociation.

One of the most promising agents employed thus far in an attempt to bring about antigenic fixation is Iodine. Quantitative estimation has shown that living pneumococci suspended in a standard

solution of iodine absorb surprisingly large amounts of the reagent. After saturation with iodine the cells are killed and remain intact, well formed and Gram positive for months. They undergo no autolysis and the processes of oxidation cease. The inactivation of lytic enzymes, the suppression of oxidative reactions and the preservation of morphological integrity are of distinct advantage in conserving the antigen.

Comparison of heat killed and iodized cells shows that in the former instance, death resulting from exposure to 56° - 60°C. is soon followed by cell disintegration with the appearance of shrunken, Gram negative and shadow forms, due in part perhaps to incomplete inactivation of cellular enzymes at this temperature and in part to extraction which subsequently goes on in the saline suspension. On the other hand, the unheated, iodized cells prepared from the same mass culture remain intact and retain indefinitely their form, size and staining reactions. Moreover, the iodized vaccine is non-toxic, and animals treated for weeks with these preparations show no ill effects.

Immunological investigations by other workers have demonstrated that iodized proteins are altered in their antigenic specificity. The type-specificity of pneumococcus antigen, however, is determined not by the bacterial protein but by the carbohydrate substance which envelopes the cell. Moreover, the polysaccharides of the three specific types differ from the starch-glycogen group of carbohydrates in giving no color reaction with iodine. Such combination as iodine makes with the cell, therefore, does not chemically alter this important and determinative constituent of the antigen. That the specificity of the iodized cell remains antigenically unaltered is shown by

the fact that serum of immunized animals agglutinates equally well both the treated and untreated organisms and protects mice against infection with virulent organisms of the homologous type.

The use of formalin as fixative and the increased production of antibodies when formolized cells of Type I are used as antigen, was discussed in the preceding report. Experiments now in progress will shortly yield results whereby the comparative merits of the various fixatives for the different bacterial types may be finally determined. Apart from the interesting theoretical considerations of the relation of these observations to the actual chemical constituents of the immunizing antigen, the methods evolved may eventually come to have practical value in the production of potent antisera for therapeutic purposes.

II. Autolysis. (Dr. Goebel)

As part of the problem of determining the nature and chemical structure of the immunizing antigen of pneumococcus, a study is being made of cell autolysis. During spontaneous dissolution of pneumococci there is an accompanying dissociation by the effective type-antigen. Therefore, to know whether proteolytic and lipolytic cleavage of cell substances occurs during autolysis, and to what extent, if any, these changes are associated with the loss of antigenicity of the autolysate, would, indirectly at least, afford some clue to the mechanism of antigenic dissociation and to the nature of the linkage between the carbohydrate and the substance which confers antigenicity upon it.

From the experimental evidence already available, it is apparent that autolysis is not to be confused with proteolysis,

since only slight increase in amino nitrogen occurs and the cell protein after prolonged autolysis still retains its antigenicity. The ratios of total nitrogen to non-coagulable and amino nitrogen are also being determined since it is known that during autolysis some degradation product is released, probably in the form of a proteose, which gives rise to the purpura producing substance in pneumococcus autolysates.

So far, little or no appreciable hydrolysis of the lipoidal substance of the cell has been determined, although final judgment on this phase of the autolytic process must await the use of the more exact methods now being perfected.

The outcome of these studies on autolysis, whether they throw light on the processes of antigenic dissociation or not, will at least add to the knowledge of the action of the intracellular enzymes on the native constituents of the cell itself and supplement our earlier studies on the action of these same ferments on foreign substrates.

III. Bacterial Variation.

1) Pneumoccccus.

a. Interconvertibility of "R" and "S" forms. (Dr. Dawson)

(Conversion of "S" to "R".) The process of conversion of virulent, type-specific, capsulated "S" types of pneumococcus into avirulent, non-type specific, non-capsulated "R" forms has been studied in further detail. The most effective means of bringing about this change is by growth in homologous immune sera and differences have been found to exist in the readiness with which the transformation can be effected in the various types. Type I "S" has been found

to be the most difficult to convert to the "R" forms requiring ten to fifteen transfers in serum dilutions of 1:2 or 1:4. Even after this number of transfers "intermediate" colonies exist which readily revert to the "S" type. On the other hand, Types II "S" and III "S" are readily converted to the "R" variety, and in each instance the change is more abrupt and complete than in the case of Type I "S".

(Reversion of "R" to "S"). Reversion of "R" forms to their homologous "S" type has been effected both by an in vivo and an in vitro method. (a) In Vivo. By animal passage "R" forms, derived respectively from Types I, II, and III "S", have been transformed into the "S" type. Differences have been found to exist in the constancy of the "R" variant. Some "R" forms readily revert to the virulent "S" type while one strain has been studied which has remained completely refractory to all attempts to effect the transformation.

In the earlier experiments the intraperitoneal route was used exclusively but more recently the subcutaneous injections of large amounts of "R" cultures has given a much greater number of positive results. Single-cell as well as mass cultures have been employed in all experiments and in every instance the single cell cultures have reacted in the same manner as the mass cultures from which they were derived. (b) In Vitro. The in vitro method of effecting the "R" -+ "S" transformation is by growth of the "R" forms in anti-"R" sera, the optimal concentration of which is 10 per cent. As in the in vivo experiments both mass and single-cell cultures were employed. Such a finding argues against the hypothesis that the virulence of a culture depends upon the relative number of "R" and "S" forms of which it is composed.

In all instances in which reversion has been effected both in vivo and in vitro the "R" forms have reverted to the "S" type from which they were originally derived.

Reversion of "R" to "S" is always accompanied by the acquisition of all the characteristics of the "S" type, including maximal virulence.

2) Effect of Adaptation to Growth at Higher Temperatures.

Preliminary observations have been made on the growth of Types I and III pneumococcus at higher temperatures (39°C). The results of early investigations have been such as to suggest that further work may reveal significant differences in the behavior of the various types. After fifteen transfers at 39°C the strain of Type I "S" employed had completely changed to the "R" form, while after thirty transfers at the same temperature a Type III "S" strain still produced only "S" colonies. Further work is now being undertaken to determine the effect of growth at 39°C on a variety of serological types.

3) Friedländer's Bacillus. (Dr. Julianelle). During the course of studies on the biological and immunological properties of Friedländer's bacillus, three sharply defined types were found to exist among different strains of this organism. The types were designated A, B, C, and into one Group, X, were placed several heterogenous strains. Later studies revealed that, under certain conditions, variant forms may be induced in cultures of Friedländer's bacillus. The typical colonies of the organism are identified as Smooth (S) and the variant colonies as Rough (R). The "S" strains produce capsules, soluble specific substance, are virulent and type specific.

The R strains, on the other hand, produce no capsules, nor soluble specific substance, are not pathogenic and are group specific; that is, they are serologically undifferentiated regardless of their type derivation.

Further study has since disclosed that there exist among the "R" variants of Friedländer's bacillus additional forms which exhibit definite differences in morphology and antigenicity. Three different forms of R colonies have been studied and designated as R_1, R_2, R_3. The R_3 variety is extremely unstable and since it was never obtained in pure culture, it was studied only morphologically. R_1 and R_2, however, have been observed in greater detail and they have been found to differ both grossly by colony formation, and microscopically by the size and arrangement of the individual cells. Both variants (R_1 and R_2) moreover, may be differentiated further by serological reactions. Both forms are agglutinated in antisera prepared by the injections of rabbits with either strain, but they lack the capacity of complete reciprocal agglutinin adsorption. Each variant adsorbs from the homologous antiserum agglutinins for both homologous and heterologous organisms; from the heterologous serum, however, antibody is removed only for the strain employed in the adsorption. The R forms differ considerably from their antecedent "S" strain in colony appearance morphology, virulence and antigenicity.

A number of methods have been adopted to induce reversion of the R forms to the parent "S" type. However, whether the technique or its application was inadequate, the results were uniformly negative. This does not mean that all "R" cells of Friedländer's bacillus are irreversible, but that under the conditions of the

experiment, the method employed did not supply the proper stimulus to reversion.

The spontaneous development of "R" variants in "S" culture has been found to accompany the process of aging. Growth in homologous immune sera in vitro also converts the "S" cells into "R" forms. That variation, however, is more than in vitro or cultural degradation gains support from the fact that "R" forms have been found in cultures taken directly from foci of infection in the animal body. In fact, in 17 cultures from different sources "R" forms were found in 5 instances. Interestingly enough, the "R" variants were found only in chronic infections and always in conjunction with "S" forms.

Further Observations on the Occurrence of Specific Types of Friedländer's Bacillus in Disease. (Dr. Julianelle). In the preceding report, a summary was presented of the distribution and relative frequency of occurrence of specific types in infections associated with Friedländer's bacillus. Up to that time, observations were made upon 39 strains; since then, the number has been increased to 68. In the following table the distribution of the specific types is summarized to date.

Total number of Strains studied	Type A	Type B	Type C	Group X
62	32	9	7	13

Type A - 32 strains.

24 strains from Pneumonia in man, 2 from adenoid tissue, 2 from throat cultures, 2 from fatal abscesses in guinea pigs, 1 from liver abscess in man, 1 from cystitis.

Type B - 9 Strains.
4 strains from fatal Pneumonia in guinea pigs, 3 from Pneumonia in man, 2 from genito-urinary infection in horses.

Type C - 7 Strains.
2 strains from Pneumonia in man, 1 from sputum of Pneumococcus pneumonia, 1 from subacute sinusitis of antrum, 1 from nose (sinus infection), 2 source unknown.

Group X - 13 Strains. 6 strains from Pneumonia in man, 2 from fatal infection in guinea pigs, 1 from cystitis, 1 from feces (Pellagra), 1 from liver abscess in man, 1 from lung abscess, 1 from throat culture.

Although the total number of strains of Friedländer's bacillus studied is still insufficient to furnish conclusive data on the relative frequency and distribution of types, nevertheless, it is interesting to observe that of 35 strains isolated from pneumonia in man, 29 or 83 per cent belonged to one or other of the three fixed types. The frequency of Type A infection in Friedländer's pneumonia in man is shown by the fact that organisms of this type were isolated from 24 (68 per cent) of 35 cases studied. It is remarkable that these percentages are the same now with a total of 62 strains, as they were upon the former analysis based on 39 strains.

Since there seems to be great confusion as to what definitely constitutes a Friedländer's bacillus, and what sugars are fermented by organisms of this group, the reactions of 45 strains have been studied in media containing the different carbohydrates, dextrose, lactose, sucrose, mannite and maltose.

The results of the fermentation studies reveal that, in general, there is great variability among the different strains and that the adaptation of such reactions for a classification of this species offers more confusion than system. The fermentation reactions not only do not aid in the classification of types but offer no assistance in differentiating the Friedländer's bacillus from closely allied organisms. The one striking fact brought out in this study is that whereas the majority of strains in Types B and C and Group X ferment lactose, 61 per cent of the strains of Type A produce neither acid nor gas from lactose.

IV. Natural Resistance and Acquired Immunity to Pneumococcus. (Dr. Tillett)

In three previous papers, experimental studies on natural and acquired immunity of rabbits against pneumococcus mucosus have been described. Since an interpretation of the results reported in the published experiments has formed the basis of subsequent work, a brief review of the earlier studies will be given.

From observations on the results of injection of Type III pneumococci into normal rabbits, two main facts have accrued which seem to be related. First, it was found that immunization of rabbits with heat killed Type III vaccine or living organisms failed to elicit specific antibody response; secondly, that injection of living, encapsulated, mouse-virulent, Type III organisms in large doses into rabbits did not produce fatal infection. These two results led to the development of the hypothesis that normal rabbits possess some mechanism which is capable of severely injuring the capsular component of the Type III bacterial cell. On the basis of this conception the absence of type specific agglutinins in the serum of immunized rabbits is explained as

being due to the dissociation of the type specific component of the antigen; when living pneumococci are injected, injury of a similar character is inflicted on that part of the cell which is intimately associated with virulence, namely, the capsular fraction, and, in this instance, recovery of the infected animal is brought about. In the previous published experiments, which concerned acquired resistance it has been shown that rabbits when immunized either with non-type specific, avirulent, R forms of pneumococci or with the fixed types themselves, are resistant to infection with a strain of Type III, which had been made virulent for rabbits. It has also been subsequently found that immunization with R forms of pneumococci induces active immunity in rabbits against infection with Type I or Type II organisms as well as Type III. Since when R organisms are used for immunization the production of type specific antibodies is excluded, it has been necessary to seek elsewhere for a correct interpretation of this form of active immunity. It has seemed possible that the factors of natural resistance previously discussed might play a part in this non-type specific immunity and that the increased resistance of rabbits immunized with R pneumococci might represent an exaltation of the mechanism normally present. Consequently, the work of the past year has been devoted to a study of this form of specific immunity. It seemed of first importance to determine whether the acquired immunity was due to protective substances present in the circulating blood, in the fixed tissues, or both. Consequently, experiments have been carried out to determine whether this form of resistance may be passively transferred to normal animals by means of the blood or serum of resistant rabbits. Prev-

ious attempts to protect mice passively by the use of serum of rabbits immunized with R pneumococci have been uniformly negative. Repetitions of protection tests of this kind using both whole blood and serum in relatively large amounts and varying the time of serum injection with respect to time of infection, have always resulted in failure. However, in contrast to the failure of passive transference in mice, it has been found that the blood and serum of anti-R rabbits does passively protect normal rabbits to a marked degree against infection with any of the fixed types of pneumococci. The transference of protection, successfully accomplished by the use of whole citrated blood and by serum, demonstrates that the protective substances are present in the circulating blood. Following the establishment of this fact, it became necessary to determine how much blood or serum was required to furnish maximum protection; the degree of protection in terms of the infecting dose of culture; and the duration of the protection this afforded. It has been found that 15 to 20 cc. of blood or 10 to 15 cc. of serum afford greatest protection and that this amount constantly bestows a solid immunity against 10,000 lethal doses and in many instances against 100,000 lethal doses of virulent pneumococci of any type. It has also been determined that the passive protection afforded by whole blood endures for as long as three weeks after transfusion - longer periods have not been tested.

Experiments planned to define more clearly the nature of the protective substances in the blood of rabbits immunized with R organisms are at present in progress but the results are not as yet complete enough to warrant reporting. The nature of the investiga-

tions at present under way include attempts to absorb from immune serum, by means of pneumococcal cells, the active principles; to neutralize them with specific carbohydrates derived from pneumococci; to determine their heat lability; to determine their influence on growth and viability of the organisms in vitro. It is a striking fact that although immunization of rabbits with degraded, avirulent pneumococci stimulates no demonstrable type specific antibodies, these animals possess a high degree of active immunity to virulent pneumococci of all types. Furthermore, the blood of rabbits so immunized although unable to protect mice, does afford passive protection to normal rabbits. Work is being carried on to define more clearly the actual nature of this form of acquired resistance which is effective in the absence of type specific antibodies and which appears to involve principles other than those usually associated with type-specific immunity.

V. Specific Anaphylaxis with Pneumococcus Polysaccharide. (Dr. Tillett)

Previous studies on bacterial anaphylaxis with Pneumococcus have dealt with the action of cell solutions and autolytic extracts containing unavoidable admixtures of protein and other soluble products which are often primarily toxic. As the result of recent chemical studies, there are now available in highly purified form specific carbohydrate derivates of Pneumococcus which are devoid of toxic properties and free from protein degradation products. In view of the increasing significance of these specific polysaccharides in the processes of immunity, it seemed important to determine whether these chemically purified and protein-free substances isolated from type strains of pneumococci

actively participate in the reactions of bacterial anaphylaxis.

The results may be briefly summarized as follows: Pneumococcus polysaccharides isolated in protein-free form from Pneumococcus Types I, II, and III are devoid of the function of inducing active anaphylactic sensitization in guinea pigs. This lack of sensitizing power adds further evidence in support of the view that these substances, when chemically purified, are devoid of true antigenic function. The fact, however, that in the dissociated form they retain, as haptens, the property of combining specifically with antibacterial antibodies led to attempts to test their capacity to induce anaphylactic shock in passively sensitized animals. Guinea pigs may be rendered passively anaphylactic to the specific carbohydrates with the precipitating sera of immune rabbits. Animals passively sensitized in this manner die in three to four minutes with acute shock following an injection of amounts as small as 0.005 mg. of protein-free polysaccharides of the homologous type. The symptomalogy and pathology of the animals are identical in every way with those of true protein anaphylaxis. The reactions are type-specific.

VI. Are the Specific Precipitable Substances of Pneumococcus, Haptens? (Dr. Julianelle).

This is the title of a paper recently published by Schiemann and Casper of the Koch Institute in which, on the basis of their experimental results they answer this question in the negative. They isolated carbohydrate substances from the specific types of pneumococci and purified them by chemical methods until they no longer gave the protein color tests. In one instance, by

repeated purification the material was rendered nitrogen-free. They found that mice injected with these preparations in repeated small doses, acquired a considerable degree of active immunity against infection with virulent pneumococci of the homologous type. By reason of the immunity induced, they conclude that the specific precipitable substances are true antigens and not haptens.

These results were apparently so definite, and at the same time so contrary to our own conception of the immunological nature of the specific polysaccharides, that confirmation of one or the other point of view seemed essential.

Fortunately, we had at our disposal highly purified preparations of the specific carbohydrates which by chemical test were known to be free of protein and in the case of the Type II and Type III substance to be also nitrogen-free. The Type I substance differs from the other two in containing nitrogen as an apparently essential component of a nitrogenous sugar compound; despite the occurrence of nitrogen in this preparation it fails to give any of the protein color tests.

To test the antigenic and immunizing power of these substances, mice were given five injections intraperitoneally at four day intervals of a solution containing weighed amounts of carbohydrate of each of the three types. The total amounts of substance given the various groups of mice comprising 96 animals in all, ranged from 5 mgs. to 0.5 mg., which in terms of bacterial equivalent represents a large amount and a wide range of dosage. Nine days after the last injection the animals were infected with graded doses of virulent pneumococci of the corresponding types.

The results of these experiments were decisive. Under the conditions employed, repeated injections of the purified, protein-free polysaccharides failed to induce in mice any measurable immunity against infection with virulent pneumococci; the animals died of an overwhelming septicemia following an infecting dose as small as 0.000001 cc. of virulent culture.

These results, together with the previous failure to demonstrate the presence of antibodies in the serum of rabbits repeatedly injected with larger amounts of these substances, and our recent failure to induce active sensitization in guinea pigs after the administration of relatively enormous amounts of those same preparations, convince us of the validity of the views that pneumococcus polysaccharides when sufficiently purified are non-antigenic substances which conform in all respects to the immunological principles governing haptens in general.

VII. Immune Response of Rabbits to intracutaneous Vaccination. (Dr. Julianelle).

Rabbits have been injected intracutaneously at weekly intervals with suspension of heat killed pneumococci and the character of the local reaction, the antibody response and the development of active immunity have been followed over a considerable period in a large series of animals. The injection of the dead bacterial bodies into the skin gives rise to a local reaction which increases in size and intensity for the first four or five injections, then gradually diminishes but never completely disappears. Accompanying these reactions the rabbits acquire a marked resistance to intravenous injection of virulent, living organisms; the active immunity

acquired in this manner is sufficiently solid and broad to protect the animal against infection with heterologous types of pneumococci. This form of actively acquired immunity, moreover, is built up in the absence of demonstrable type-specific antibodies; for rarely do rabbits vaccinated by the intracutaneous route, even after prolonged treatment, develop detectable traces of type-specific immune substances in their blood. This is the more striking since in the case of pneumococcus, Type I, at least, comparable amounts of heated cells injected intravenously, invariably stimulates the formation of type specific antibodies in readily demonstrable quantities. During cutaneous vaccination, but often only late in the process, the so-called secondary antiprotein antibodies appear in considerable titre in the blood. However, antibodies of this variety are unrelated to the type of pneumococci used in immunization and represent the response to bacterial protein without references to type specificity. Although animals cutaneously vaccinated are themselves actively immune to infection, their serum, in the absence of type-specific antibodies, fails to confer passive protection on mice against infection with pneumococci.

The relation of these results to prohpylactic vaccination, to bacterial allergy and acquired resistance offers an interesting field for further investigation.

VIII. Studies on Oxidation and Reduction by Pneumococcus. (Dr. Dubos)

1) Bacterial Antagonism. The antagonistic action of Pneumococcus upon the growth of Staphylococcus aureus was first noted by Alivasatos who recorded his observations without attempting to explain the mechanism, since none of the reasons usually ascribed adequately explained the phenomenon he observed. Whenever pneumococci

and staphylococci are seeded together on the surface of an ascitic agar plate, the latter organisms fail to develop and are often completely suppressed in the areas whereas growth of pneumococcus is most abundant.

It is known that during aerobic growth, pneumococcus cells combine with oxygen and that as a result of this union there is formed a substance, having the reactions of hydrogen peroxide, which accumulates in considerable concentration in the medium. The presence of this agent in aerobic cultures has in earlier experiments been shown to account for many of the phenomena associated with the life and death of the bacterial cell. The formation of methemoglobin, the destruction of the endocellular hemolysin, the inactivation of the cell enzymes, are in each instance reactions dependent upon oxidation processes, brought about through the action of pneumococcus peroxide.

It has now been demonstrated that the mechanism of the bacterial antagonism between pneumococci and staphylococci is directly related to the presence and accumulation of this same peroxide, to the toxic action of which staphylococcus is particularly susceptible. Proof that the mechanism is one of cellular oxidation lies in the fact, that these same two species, between which there exists such marked antagonism on aerobic cultivation, grow together in perfect symbiosis when air is excluded from the culture or when a catalyst is added to the medium; that is, under conditions which inhibit the formation or prevent the accumulation of peroxide.

2) Relation of Oxidation processes to growth and viability of Pneumococcus. In recent experiments it has been found that the

processes of cellular oxidation markedly influences the death rate of pneumococci during artificial cultivation. Cells suspended in broth or buffer solution exposed in shallow layers to the action of air form large amounts of peroxide and died within 24 hours. The addition to these bacterial suspensions of substances, such as yeast extract which are known to activate oxidation reactions, increases the formation of peroxide and lessen the viability of the cells; while on the other hand, the mere addition of organic or inorganic catalase, in the form of plant tissue or active iron salts, prevents the accumulation of peroxide and correspondingly increases the viability of the cells. In the latter instance, the cells are protected almost as effectively as if they were placed under anaerobic conditions and the bacteria remain viable for weeks.

The influence of oxidation processes upon cell multiplication and growth is being studied particularly with reference to the effect upon the initiation of growth in media of different oxidation - reduction potentials.

IX. Nature and Duration of Immunity Induced by Inhalation Method. (Dr. Stillman).

Studies on the nature of the immune response of rabbits to repeated inhalations of living pneumococci have been continued. It has already been shown that the serum of rabbits which have been repeatedly sprayed with Type I pneumococci developed in their serum agglutinins and protective antibodies. After the inhalations of pneumococci are discontinued, the agglutinins rapidly disappeared. The protective antibodies, however, persist for long periods. The sera of certain rabbits, which survived treatment and are living 640 days after their last exposure to an atmosphere of sprayed pneu-

mococci, still continue to protect mice against infection with virulent pneumococci.

In order to compare the nature and duration of the immune response of rabbits sprayed with living pneumococci with that of animals vaccinated by different routes with dead organisms, a series of rabbits were injected with graded amounts of heat killed pneumococcus Type I, intravenously, intraperitoneally, intramuscularly, and subcutaneously. The sera of 80 per cent of the intravenously vaccinated rabbits contained agglutinins and all showed protective antibodies. The sera of 60 per cent of the intraperitoneally vaccinated rabbits contained agglutinins and all showed protective antibodies. The sera of 33 per cent of the intramuscularly vaccinated rabbits contained agglutinins and 86 per cent also showed protective antibodies. None of the sera of the subcutaneously vaccinated rabbits contained agglutinins although protective antibodies were present in 71 per cent. From these experiments it would appear that although there is a close relationship between the presence of agglutinins and protective antibodies in a given immune serum, they do not run parallel.

The duration of immunity following vaccination is also being studied. It is apparent that the duration of immunity bears a close relationship to the amount of vaccine administered. Whereas, the serum of a rabbit which has received but 1 cc. of vaccine passively protects a mouse against 0.01 cc. of a virulent pneumococcus 10 days following the last vaccination, after the lapse of 30 days more, the serum of this rabbit has lost its capacity to confer passive protection. On the other hand, the sera of rabbits which received

12 or 15 cc. of vaccine, after an interval of as long as 570 days, still continue to confer upon mice a high degree of protection.

The duration of the immunity in rabbits actively immunized by the inhalation method and by vaccination is being followed.

The immunological response of rabbits sprayed with other types of pneumococci is being studied. The results, thus far, seem to indicate that the character and duration of immunity induced by these methods varies with the type of pneumococci used.

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