STUDIES ON IMMUNOLOGICAL RELATIONSHIPS AMONG THE PNEUMOCOCCI.

III. RELATIONSHIP BETWEEN A VARIETY OF SACCHAROMYCES CEREVISIÆ AND THE TYPE II VARIETY OF DIPLOCOCCUS PNEUMONIÆ (PNEUMOCOCCUS).

By JOHN Y. SUGG and JAMES M. NEILL, Ph.D.

(From the Department of Bacteriology and Immunology, Vanderbilt University Medical School, Nashville.)

(Received for publication, October 18, 1928.)

INTRODUCTION.

The present paper reports evidence of an immunological relationship between one variety or type of the yeast Saccharomyces cerevisiæ and the Type II variety of Diplococcus pneumoniiæ (Pneumococcus). The immunological interrelationships among the different yeasts have never been well established, but it is certain that all of the yeasts included by the species term Saccharomyces cerevisiæ are not immunologically identical. Only one strain or variety was used in most of our investigation. Experiments were made with a number of the recognized varieties of the species, that differ from each other in fermentation and other cultural properties, but the present data are not sufficient to furnish definite proof of the possession or lack of serological relationship of these other yeast varieties to Type II pneumococci. It is necessary, therefore, to limit the present paper to the relationship exhibited by one variety of Saccharomyces cerevisiæ that was derived from commercial (Fleischmann's) dried yeast.

The immunological relationship between the yeast and the Type II pneumococcus may be reported by the presentation of two kinds of data: (1) the reactions of the yeast with Type II antipneumococcus serum; (2) the reactions of Type II pneumococci with the antiyeast serum. In the following report the reactions of the yeast with the antipneumococcus serum are presented first, although the evidence
obtained from that source is less convincing than that obtained from the reactions of the pneumococci with the antiyeast serum.

EXPERIMENTAL.

Methods.—All tests for agglutination of the yeast were made with glucose meat extract broth cultures of a yeast strain derived from a single cell culture isolated from Fleischmann's dried yeast by Dr. Roy C. Avery (1). The antiyeast immune sera were produced by immunization of rabbits with two sorts of material: (1) washed yeast cells from glucose agar cultures of the pure single cell strain; (2) the dried yeast cells contained in the commercial preparation. The yeast suspensions were of approximately equal turbidity and were heated for 5 to 10 minutes at 100°C. The immunization consisted of one to four courses of 6 daily intravenous injections with a rest period of 7 to 10 days between courses; with most rabbits, test bleedings were made at the end of the rest period following each course of injections.

The immunization with the dried yeast produced the more potent antiserum and in fact, antipneumococcus agglutinins were obtained only from rabbits immunized with the dried material. A possible objection that might be raised to basing the antipneumococcus relationship upon these antiyeast sera is answered by the fact that the antiserum produced by immunization with the dried yeast can be exhausted of antibodies reactive with either pneumococci or with yeast by absorption with yeast cells derived from the pure single cell culture alone.

The production of the antiyeast sera was complicated by the death of many of the rabbits during the immunization. The yeast suspensions did not seem to be primarily toxic for no rabbits were killed by the first course of injections. During the second and third courses (after rest periods of 7 to 10 days) a number of rabbits were killed with anaphylactoid symptoms upon injection of the yeast. While the mechanism of the reaction was not studied intensively, it was the second or third rather than the first injection following the rest period which caused the death of most of the rabbits. It is possible that the death of the rabbits was due to a true anaphylactic reaction that occurred from the second or third injection when the circulating antibodies had been removed by the first injection following the rest period. The anaphylactic or anaphylactoid deaths were more frequent with animals injected with the yeast cells from the agar cultures than with animals injected with the dried yeast.

A means of decreasing the likelihood of anaphylactic or anaphylactoid death of rabbits during immunization was observed during the latter part of the investigation. Since the death of animals utilized for production of antiserum is a serious complication in any investigation, it seems desirable to report the procedure as a possible means of preventing the acute death of rabbits during the later stages of their immunization with other kinds of antigens. At the suggestion of Dr. Walter E. Garrey attempts were made to save the life of the rabbits by intravenous in-
jection of glucose solution. A number of times, rabbits which were exhibiting marked symptoms and which indeed were apparently about to die, recovered rapidly when approximately 5 cc. of 10 per cent glucose was injected intravenously. Intraperitoneal injection of the same amount of glucose was also given to most of these animals immediately or soon after the intravenous injection.

Agglutination of the Yeast by Type II Antipneumococcus Serum.

1. Agglutination by Type II Antipneumococcus Immune Horse Serum.—The tests were made with 3 different Type II antipneumococcus, 7 anti-Type I, 1 anti-Type III, and 4 antimeningococcus immune horse sera. When observed after 2 hours at 37°C., the yeast was agglutinated only by anti-Type II serum. The agglutination occurred with undiluted and one-fifth dilution but not with one-tenth dilution of serum. None of the other horse sera showed any agglutination. The distinction became somewhat less clean-cut after ice box storage which resulted in slight agglutination of the yeast cells in four of the eleven control horse sera; this occurred only in undiluted serum and was never comparable to that in any of the three anti-Type II immune horse sera. These results with the antipneumococcus serum from horses appeared to furnish definite evidence of a serological relationship between the Type II pneumococcus and the yeast.

2. Agglutination by Immune and by Normal Rabbit Serum.—In the experiments in which the relationship between the yeast and the Type II pneumococci was studied by tests of yeast agglutination with Type II antipneumococcus rabbit serum, it was found that the serum of many normal rabbits possessed the property of agglutinating the yeast. While these experiments contributed no proof of relationship between the yeast and the Pneumococcus, the results summarized in Tables I and II are important as experimental facts concerning the irregularities in the yeast-agglutinating capacities of serum from different individual rabbits.

Table I presents the results of agglutination tests made with several bleedings from 168 different rabbits. It is evident that the yeast was definitely agglutinated by the serum of some, but not by the serum of other rabbits. Due to the number of animals tested, the most satisfactory method of presenting the results is in the form of the percentage of animals whose serum agglutinated the yeast in the different test dilutions. In tests with undiluted, one-fifth, and one-tenth dilutions of serum, there was no significant difference in the percentage of yeast
agglutinators between the group of 33 rabbits immunized with Type II pneumococci, the group of 75 immunized with other bacteria, or the group of 60 rabbits that had not been immunized at all. The fact that serum from a few of the rabbits immunized with pneumococci agglutinated the yeast in higher dilutions than did serum from any of the other rabbits must be considered of little significance in the absence of tests with serum obtained from bleedings of the same animals before their immunization. While impossible to test normal bleedings of these rabbits, there were available two or three bleedings taken during different stages of the antipneumococcus immunization of almost all the pneumococcus group of rabbits. Repeated tests of the early and late bleedings of these rabbits showed

TABLE I.
Comparison of Yeast-Agglutinating Property of Serum from "Normal" and Immunized Rabbits.

<table>
<thead>
<tr>
<th>Number in group</th>
<th>Previous immunization</th>
<th>Percentage of animals whose serum agglutinated yeast in different dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dilution of serum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Undil.</td>
</tr>
<tr>
<td>33</td>
<td>Type II pneumococci</td>
<td>42</td>
</tr>
<tr>
<td>39</td>
<td>Type I and III pneumococci</td>
<td>40</td>
</tr>
<tr>
<td>36</td>
<td>Diphtheria, anthrax, or tetanus bacilli</td>
<td>36</td>
</tr>
<tr>
<td>60</td>
<td>&quot;Normal&quot; or non-immunized</td>
<td>43</td>
</tr>
<tr>
<td>168</td>
<td>Total immune and normal</td>
<td>40</td>
</tr>
</tbody>
</table>

* Per cent of individuals in respective group whose serum agglutinated yeast cells; i.e. \( \frac{\text{Number of yeast agglutinators}}{\text{Number of rabbits in group}} \).

no significant differences in the yeast-agglutinating titre of the different samples. Since the antipneumococcus agglutinins did increase between the early and late periods of immunization of the same animals, it seems probable that the injection of the rabbits with Type II pneumococci had little if anything to do with the titre of yeast agglutinins.

Table II consists of a summary of tests of the persistence of the yeast-agglutinating property of the serum of individual "normal" rabbits. Serum was obtained from 24 non-immunized rabbits by two bleedings separated by an interval of 6 weeks, and the two samples from the same rabbits tested against the yeast culture. All the rabbits whose serum possessed yeast agglutinins at the time of the first bleeding, likewise possessed them 6 weeks later; and none of the rabbits lacking
yeast agglutinins at the time of one of the bleedings possessed them at the time of the other bleeding. Moreover, the actual titre of the yeast agglutination was surprisingly constant for each individual rabbit, a significant difference in the titres of the two bleedings from the same animal being found with only 4 of the 24 rabbits of the series. This uniformity in the lack or possession of the yeast-agglutinating capacity over a period of 6 weeks is of some significance as proof that the agglutinating property is a characteristic of the individual rabbit, that it is not gained or lost in short periods of time. The so called "normal agglutinins" which are frequently encountered in tests against many kinds of bacteria are always difficult to explain. In the present instance, there is no knowledge of either the nature or of the origin of the property responsible for agglutination of the yeast. It is possible that the agglutination by serum from one "normal" rabbit and not by that from another rabbit, is due to some non-specific constituent

**Table II.**

*Relative Constancy of the Yeast-Agglutinating Property of the Serum of Individual "Normal" Rabbits from 2 Bleedings Separated by an Interval of 6 Weeks.*

<table>
<thead>
<tr>
<th>Animals showing no change</th>
<th>Animals showing slight change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titre of both samples of serum</td>
<td>Total number of animals</td>
</tr>
<tr>
<td>Serum dilution</td>
<td>1/5</td>
</tr>
<tr>
<td>No agglutination</td>
<td>14</td>
</tr>
</tbody>
</table>

or physical property that varies in different individual rabbits. It is not impossible, however, that the yeast agglutination may be due to specific antibodies arising from some unrecognized chronic infection of individual rabbits with microorganisms possessing a serological relationship to the yeast.

*Agglutination and Protection Tests with Type II Pneumococci against Antiyeast Serum.*

The tests of the yeast cells against Type II antipneumococcus serum from horses had furnished some evidence of serological relationship between the yeast and the Type II pneumococci. More convincing evidence of the relationship was furnished by the reactions of the Type II bacteria when tested against the antiyeast serum. The degree of reactivity of the antisera from two of the rabbits immunized
with yeast was comparable to that of the antisera often obtained by immunization of rabbits with Type II pneumococci themselves; broth cultures of Type II pneumococci being agglutinated by 1/40 dilution of the antiserum and mice being protected against $1 \times 10^{-3}$ cc. of broth cultures of the Type II bacteria. There was considerable variation in the antipneumococcus value of the serum obtained from different rabbits immunized with the yeast. Some did not agglutinate broth cultures of the pneumococci, but the serum of all seven rabbits which survived immunization with dried yeast did contain some antibodies reactive with Type II pneumococci as indicated by their specific passive protection of mice against at least $1 \times 10^{-4}$ or $1 \times 10^{-5}$ cc. of virulent Type II pneumococci.

### TABLE III.

**Absorption of Antiyeast Immune Serum with Yeast Cells and with Types I, II, and III Pneumococci.**

<table>
<thead>
<tr>
<th>Antyeast serum absorbed with</th>
<th>Yeast agglutination</th>
<th>Type II pneumococcus agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum dilution</td>
<td>Serum dilution</td>
</tr>
<tr>
<td></td>
<td>1/5</td>
<td>1/320</td>
</tr>
<tr>
<td>Yeast cells</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Type II pneumococci</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Type I pneumococci</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Type III pneumococci</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Unabsorbed</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

Pneumococcus Type Specificity of the Antiyeast Immune Sera.

While the antiyeast sera contained antibodies reactive with Type II pneumococci, they were apparently devoid of antibodies reactive with either Type I or Type III. The same antisera that agglutinated and protected against different strains of the Type II variety, always proved entirely non-reactive with the other types of pneumococci. This is an important index that the relationship of the yeast to the Type II pneumococcus is an S-anti-S relationship which does not extend to other serological varieties of pneumococci.
Absorption of Antiyeast Immune Sera.

Antiyeast immune (rabbit) serum was absorbed with suspensions of heated yeast cells and with suspensions of heated Type II pneumococci. For controls on the pneumococcus type specificity, the same antiyeast sera were absorbed with suspensions of Type I and of Type III pneumococci. The results of the several experiments made were the same. As shown in Table III, absorption of the antiyeast serum with the homologous yeast cells completely exhausted it, not only of agglutinins for the yeast but also of agglutinins for Type II pneumococci. On the other hand, repeated absorption with the Type II pneumococcus cells removed only the anti-Type II agglutinins and had little, if any, effect upon the yeast-agglutinating capacity of the serum. In contrast to the exhaustion of the Type II agglutinins by absorption with the Type II bacteria, absorption with Types I and III pneumococci did not affect the Type II-agglutinating capacity of the antiyeast immune sera.

Absorption of Type II Antipneumococcus Immune Horse Serum.

Reciprocal absorption experiments were made with Type II antipneumococcus immune horse serum by absorbing the Type II antiserum with suspensions of yeast and with suspensions of Types I, II, and III pneumococci. The results were analogous to those obtained in the absorption of the antiyeast serum; i.e., absorption of the anti-Type II serum with the Type II bacteria exhausted the capacity of the serum to agglutinate either Type II pneumococci or the yeast; absorption with the yeast cells removed the agglutinins for the yeast but had little, if any, effect upon the Type II-agglutinating capacity; absorption with Types I and III pneumococci had no significant influence upon the agglutination of either the yeast or the Type II bacteria. The exhaustion of the anti-Type II serum by absorption with the homologous Type II pneumococci was to be expected, but the removal of the yeast agglutinins from the antipneumococcus serum by homologous (Type II) absorption is of interest as an index that the yeast-agglutinating capacity of the anti-Type II immune horse serum is due to antibodies reactive with the Type II bacteria that were utilized in the immunization by which the antipneumococcus serum was prepared.
The results of reciprocal absorption of both the antiyeast and the antipneumococcus immune sera are the same as those usually obtained in reciprocal absorption experiments with immunologically related, but different, kinds of bacteria. The similarity in the results is an interesting example of the fact that the same immunological relationships demonstrable between bacteria are likewise demonstrable between antigens derived from microorganisms as biologically unrelated as are the yeasts and the bacteria.

DISCUSSION.

The preceding experiments dealt with the immunological properties of one variety or type of the *Saccharomyces cerevisiae* species of yeast. Evidence of immunological relationship of this variety of yeast to the Type II variety of Pneumococcus was furnished by the reactions of the yeast and of the pneumococci in the homologous and heterologous (antiyeast and antibacterial) immune sera.

The data obtainable from the reactions in the antipneumococcus serum were necessarily limited to the immune serum from horses, for the yeast-agglutinating capacity of the serum from many normal rabbits complicated the interpretation of the experiments with antipneumococcus serum from immune rabbits. However, anti-Type II serum from immune horses gave clean-cut results; although definite only in one-fifth dilution of the serum, the yeast agglutination occurred in all of the Type II horse sera tested and not under the same conditions in the serum from eleven other horses immunized with other bacteria. The evidence obtainable from the reactions of Type II pneumococci with potent antiyeast sera, is convincing, for the serum of some rabbits immunized with dried yeast cells approached the anti-Type II potency of the serum obtained by immunization with the Type II bacteria themselves. It is interesting to observe that there was no regular relation between the anti-Type II pneumococcus and the antiyeast potencies of the individual immune sera, i.e., the antiyeast sera most reactive with the yeast were not always the ones most reactive with the Type II pneumococci. The irregularity in the relation potencies (ratio of \(\frac{\text{Anti-Type II bacteria potency}}{\text{Antiyeast potency}}\)) of the antiserum from individual rabbits immunized with yeast probably
represents differences in the individual responses of rabbits to different antigenic constituents of the yeast.

There is every reason to believe that the relationship of the yeast to the Type II pneumococcus in an S-anti-S relationship. Pure solutions of the S substance from yeast or from the Type II bacteria were not available. However, a number of tests were made with the filtrates of young, unautolyzed, broth cultures of Type II pneumococci which contain the S substance and no other known serologically reactive constituent. The fact that potent antiyeast sera invariably precipitated these filtrates with the formation of the compact disc characteristic of S-anti-S precipitates indicates that the same sera would likewise precipitate solutions of the purified carbohydrate derived from Type II pneumococci. While tests with the purified substances would be desirable, one may conclude that in all probability the serological relationships evidenced between the yeast and the Pneumococcus in both the antiyeast and the antibacterial sera are due to reactions between anti-S antibodies and the specific polysaccharides of this variety of yeast and of the Type II variety of Pneumococcus. In view of the usual dependence of immunological relationship upon chemical structure, there must be some chemical likeness between the S substances of these two kinds of microorganisms. The possibility of chemical relationship between the S substances derived from the yeast and the Type II bacteria is particularly interesting since pure solutions of both these polysaccharides have been studied chemically: that of the yeast by Mueller (2), and that of the Pneumococcus by Heidelberger and Avery (3).

The relationship of the yeast to the Type II bacteria is apparently an example of heterogenetic specificity, like that of the Type II pneumococcus to some strains of the Friedländer bacillus (4). The present example is particularly interesting, however, because of the obviously distant genetic relationship of the groups of microorganisms in which the immunologically related strains occur; the one belonging to the Class of Schizomycetes, pathogenic and rarely found outside of the animal body; the other belonging to the Class of Ascomycetes, non-pathogenic and leading a free existence in nature. The more complete knowledge that is being obtained by chemical studies of the specific antigenic constituents of bacteria is furnishing further demon-
strations of the fundamental principle stated by Wells (5) in his early studies on the plant proteins, that “specificity . . . . is determined by the chemical structure . . . . rather than by the biological origin” of the reacting substances. Since the cells of each plant or animal contain a number of different antigens, there naturally are a number of possibilities for likenesses in the chemical structure of some one of the various cell constituents of phylogenetically unrelated forms of life. Frequently these chemical likenesses may be biologically fortuitous (6) and the consequent serological relationship should be considered an “accident” rather than an index of genetic relationship.

This is obviously the case with the described serological relationship between the yeast and the Type II pneumococcus, where from the standpoint of S-anti-S reactions alone, the Type II pneumococcus has more in common with a particular member of the Class of Ascomycetes than with the Type I and Type III varieties of its own species. The latter evidence their real relationship to Type II pneumococci by their mutual P-anti-P reactions, for the P is apparently the genetically significant antigenic constituent of Pneumococcus.

SUMMARY.

The paper reports evidence of an immunological relationship between one variety of Saccharomyces cerevisiae and the Type II variety of Diplococcus pneumoniae (Pneumococcus). The most convincing data consisted of the reactions of the Type II bacteria with potent antiyeast serum which agglutinated, and protected mice against these pneumococci as well as the average antiserum obtained by immunization of rabbits with Type II bacteria themselves. The reactivity of the antiyeast serum is strictly specific to the Type II variety of Pneumococcus in the sense that it is entirely devoid of antibodies reactive with Type I or III. The results of absorption experiments with both the antiyeast (rabbit) serum and the anti-Type II (horse) serum were the same as those usually obtained in analogous experiments with immunologically related, but not identical, kinds of bacteria.

The immunological relationship of the yeast and the Type II pneumococcus is apparently based upon S-anti-S reactions. It repre-
sents an example of heterogenetic specificity which is of particular interest because of the wide genetic separation of the pathogenic schizomycete and the saprophytic ascomycete.

Data on the individual irregularity in the yeast-agglutinating capacity of serum from non-immunized or "normal" rabbits are presented as experimental facts.

REFERENCES.