INVESTIGATIONS INTO THE ANTIGENIC CHARACTERS OF B. MESENTERICUS AND B. SUBTILIS

OLOF SIEVERS

Sero-Bacteriologic Institution of Helsingfors University, Finland

Received for publication, May 17, 1941

In a previous paper I reported in collaboration with Zetterberg (Sievers and Zetterberg, 1940) a number of tests performed in order to confirm, by aid of serological investigations, the biological classification of different spore-forming aerobic bacteria. The results of these preliminary experiments speak in favor of the theory that Bacillus subtilis, Bacillus mesentericus, Bacillus vulgatus, Bacillus mycoides and Bacillus cereus are characterized by different antigenic structures. Since that time Lamanna (1940), according to the literature at my disposal, subjected spores of the above mentioned species to serological investigations. He maintains that, from an antigenic point of view, the spores of these organisms differ from those of the vegetative forms. The spores of B. subtilis, B. vulgatus, B. mesentericus and Bacillus agri permitted of a serological differentiation. Also, B. megatherium and B. subtilis seem to differ in this respect from the respective closely-related species.

Howie and Cruickshank (1940) examining Clostridium sporogenes, B. cereus and B. mesentericus arrived at the conclusion that spore-antisera reacted with spores and eventually to some extent also with the vegetative forms of the relative bacteria. Antisera against vegetative cells did not react with spores.

Goldman (1939) found that a bacteriophage active against B. mesentericus was capable of influencing lytically all the examined strains of B. mesentericus, B. subtilis and Bacillus saccharolyticus, whereas B. megatherium, Bacillus petasites, Bacillus asterspurus, B. mycoides and B. cereus were not at all affected.
When continuing my investigations I used the vegetative type, as my experience indicates that a spore-free emulsion of vegetative forms may be obtained more easily than an emulsion of spores entirely free of antigen of the vegetative type.

If one proceeds along the lines suggested by me with an examination of the vegetative type, one is naturally confronted by the question whether the bacterial groups indicated above may be considered as uniform serological groups, or whether by a more minute antigen analysis they may be further divided into different subgroups. Biologically, these groups are not wholly uniform, inasmuch as e.g. different strains of *B. mesentericus* may differ from one another to some extent. Zetterberg's (1940) complement-fixation tests show that *B. mycoides* and *B. subtilis* respectively have evidently each one antigen in common, whereas *B. mesentericus* and *B. vulgatus* do not show such a characteristic antigen factor common to the whole group in question.

In my continued experiments I employed the same precipitation method with autolysate used in my previous paper. The tests, however, now comprised a larger number of strains of the type *B. mesentericus* and *B. subtilis*. These strains were also this time kindly placed at my disposal by Mrs. M. Aminoff, Fil. Cand. She is later on going to propound herself the question of the biological characters of these bacteria.

**B. MESENTERICUS**

Seven of the examined strains were used in rabbit immunization tests. As in previous experiments cultures of twelve hours' standing were given in increasing doses to the animals, which were thereupon killed and the blood was secured after the precipitation tests with autolysate of the bacteria used for the relative immunization had given a clearly positive reaction. Decreasing quantities of autolysates and undiluted antisera (autolysate: antiserum = 10:1) were employed in the experiments. The reading took place after 25 to 30 minutes at room temperature. The reacting sera used by me did not give any remarkably strong reactions (autolysates in dilutions varying between 1:4 and 1:256), but it seems to me that the specificity indicated by
the results justifies experiments of this kind. Table 1 shows the results attained with seven antisera obtained by injections of 20 different strains of \textit{B. mesentericus} and four control strains. In order to prevent the table from growing too extensive, the strength of the different dilutions was not recorded, the positive and negative reactions only being denoted by $+$ or $-$ respectively.

A glance at this table shows that the majority of strains have one antigen in common, as they all react with antisera 1740,

\begin{table}
\centering
\begin{tabular}{|l|ccccccccc|}
\hline
 & \multicolumn{9}{|c|}{\textbf{AUTOLYSATE OF B. MESENTERICUS NUMBER}} \\
\hline
 & 1741 & 1742 & 1743 & 1744 & 1745 & 1746 & 1747 & 1748 & 1749 \\
\hline
\textbf{B. MESENTERICUS} & & & & & & & & & \\
\hline
IIp11a antiserum number 1740 & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $-$ \\
III 40 antiserum number 1739 & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $-$ \\
IVp1 antiserum number 1738 & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $-$ \\
IV 2264 antiserum number 1685 & & & & & & & & & \\
IV 51 antiserum number 1678 & & & & & & & & & \\
IV 33 antiserum number 1686 & & & & & & & & & \\
IV 43 antiserum number 1749 & & & & & & & & & \\
\hline
\end{tabular}
\end{table}

1739 and 1738. The result of these experiments seems to indicate that it is here the question of one large subgroup often met with, as well as of at least three other subgroups, in this material represented by one or a few strains. The contents of antigen and albumin in the different autolysates may vary. For determining the latter content, an admixture of 0.1 ml. 20 per cent trichloracetic acid was added to 0.5 ml. of decreasing quantities of autolysate. The weakest dilution which still gave a precipitation was recorded. The figures obtained show that the albumin content
varied somewhat, and in order to get an idea whether the eventual grouping discussed above might have been influenced hereby, the final results were converted into per cent of the albumin content. This, however, did not influence in any way the serologically justified classification.

By performing a number of absorption tests I have tried to obtain further proof that the classification into subgroups indicated by the results in table 1 is correct. The absorption was carried out with cultures of 12 hours standing washed in physiologic salt solution and centrifuged. A large quantity of "dry" bacterial mass was added to the antisera. After having been kept for two hours at 37°C over night at + 4°C, the antisera were centrifuged in the usual way and tested by precipitation70: Table 2 shows the record of a test with a B. mesentericus 40 antiserum.

An absorption test with antiserum 1685 and 1686 and corresponding bacteria confirmed the heterogeneity of the grouping and composition of antigen indicated by the results of table 1.

Antiserum 1749 reacted also with the bacteria used in the immunization and with a strain (No. 44) from the largest group.
Absorption tests with this antiserum yielded the outcomes recorded in table 3.

Be it further emphasized that one strain did not react with any antiserum. An attempt to obtain antiserum with this strain (66) failed, as the serum of the rabbit did not react with the autolysate of this strain. It is impossible to prove that the autolysate did not contain any antigen. The albumin gave a positive reaction with trichloracetic acid in a dilution 1:32.

The subgrouping inferred by the first results was confirmed by these absorption tests. The outcomes show that all the B. mesentericus strains do not have any antigen in common. Neither may the four groups thus obtained be considered as a final result. Occurrence of other groups containing antigens which are perhaps foreign to the groups now observed, may in all probability be proved if more material is collected. Results indicating that representatives of the different subgroups may also have antigens in common were in one case obtained with antiserum 1749. The question whether the serologically justified classification now under discussion may have a biological parallel will be later on touched upon by Aminoff.

<table>
<thead>
<tr>
<th>B. MESENTERICUS NUMBER</th>
<th>B. MESENTERICUS 43 ANTISERUM, ABSORBED WITH THE FOLLOWING B. MESENTERICUS STRAINS:</th>
<th>IVpl1</th>
<th>44</th>
<th>IVpl1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVpl1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>44</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>43</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

B. SUBTILIS

According to Sievers’ and Zetterberg’s (1940) precipitation and complement fixation tests, B. subtilis strains have an antigen in common. The results of a repetition of their experiments with a larger number of strains and several antisera yielded the outcomes recorded in table 4. These experiments were carried out with the same technique as the corresponding tests with B. mesentericus.
The presumption that different *B. subtilis* strains have an antigen in common seems to hold good in regard to cultures now tested. That does not naturally prove that this antigen common to them all is the sole feature characteristic for this bacterium. It thus remains to try and prove by means of absorption tests, whether the strains may be also carriers of other antigens characteristic for eventual subgroups. Table 5 shows the results of an absorption test with antiserum 1742.

The fact that the strains VIIb2, 48 and 68 are not capable of completely absorbing all the components of the antiserum indi-
B. MESENTERICUS AND B. SUBTILIS

cates that these strains do not contain all the antigenic components of part-antigens contained in the strains 80 and 54. An absorption of the antisera 1743 and 1744, obtained with the strains VIIb2 and 48 succeeds not only with these two cultures, but also with the strains 80 and 54. I feel inclined to interpret this in such a way that the strains 80 and 54 at any rate ought to have, besides one or several antigens common for all the strains of B. subtilis, other antigenic characters also, i.e., possess a still more complicated antigenic structure.

Those two bacterial types now examined behaved differently, serologically. It proved possible to classify them both into subgroups, although the difference between these subgroups is not equally clearly perceptible in both these bacteria. To what an extent B. subtilis may be considered as a biologically more uniform group, will not be discussed in this connection.

SUMMARY

Sievers' and Zetterberg's (1940) experiments indicate that the vegetative forms in different spore-forming aerobic bacteria may be distinguished from one another by serological tests. A natural continuation of these investigations would open the question, whether the different groups may be considered as characterized by a uniform antigenic structure or whether the occurrence of eventual part-antigens may justify an arrangement into subgroups. The experiments were carried out according to the same technique as that employed by Sievers and Zetterberg by precipitation of autolysate.

The examined strains of Bacillus mesentericus were subdivided into four groups, one of which comprised 13 of the 20 strains examined. Three of the remaining strains could be ranged under a second group and two in a third group. One strain did not react at all and another strain had, besides its own characteristic antigen, a part-antigen in common with a strain belonging to the large group.

Contrary to B. mesentericus, all the Bacillus subtilis strains had one or several antigens in common, and some of them, besides, contained also some part-antigen.
REFERENCES

GOLDMAN, K. O. 1939 Der Bakteriophag zu Bac. mesentericus und seine Eigen-


LAMANNA, CARL. 1940 The taxonomy of the genus bacillus. J. Bact., 40,
346-359. Part II. Differentiation of small celled species by means of
spore antigens. J. Infectious Diseases, 67, 193-204. Part III. Dif-
ferentiation of the large celled species by means of spore antigens.
Ibid., 67, 205-212.

SIEVERS, OLOF AND ZETTERBERG, Bo. 1940 A preliminary investigation into
the antigenic characters of spore-forming, aerobic bacteria. J. Bact.,
40, 45-56.