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ASPECTS OF VIRULENCE IN STAPHYLOCOCCUS AUREUS
IN RELATION TO ANTIGENIC STRUCTURE*

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ABSTRACT. The author believes that the majority of recently isolated Staphylococcus aureus strains have antigen 17 or 13 and are more virulent for laboratory animals and for man than those kept in the laboratory and which have undergone a variable number of transfers. These latter have antigens 1 or 3 and are less productive as regards some or all factors responsible for virulence i.e. infectivity and pathogenicity factors. It would thus seem that antigen loss variation is simultaneously attended by weakening of other biological characteristics. Conclusions from virulence studies will only be valid if experiments have been done on strains which have not yet undergone this antigenic variation.

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In 1961 we reported the phenomenon of antigenic loss variation in Staphylococcus aureus. Recently isolated S. aureus strains usually possess two antigens, 17 or 13, but after a variable number of daily transfers from agar slant to agar slant these antigens were lost and gave place to antigens 1 or 3, respectively.

Some difficulties experienced by other authors (Brodie et al. 1958; Pillet et al. 1960; Gorrill 1965) may be easily explained in the light of this phenomenon. There have been few references to this variation in the last five years, but we believe this is because insufficient freshly isolated strains have been studied by other workers. However, our laboratory functions as a serological centre in a diagnostic routine dealing daily with fresh isolates of Staphylococcus aureus.

* Addendum No. 4 to Minutes of the second meeting (23rd July, 1966) of Subcommittee on Taxonomy of Staphylococci and Micrococci.
In our previous contribution (Torres Pereira 1965), we reasserted the general character of the phenomenon and stated that the individualization of the two antigens 17 and 13 made it possible to define two varieties of *Staphylococcus aureus*. We further suggested a relationship between antigen structure and virulence, a point which will be developed in this paper.

**Material and Methods**

*Staphylococcus aureus* strains before and after the antigen loss variation had occurred were used. For intracerebral inoculation of albino mice the following strains were selected: strains 15560 (17+17) and 15560 (17−1+); strains 43346 (17+17) and 43346 (17−1+); and strains 15128 (13+13) and 15128 (13−13).

For intraperitoneal inoculation in mice, strains 5199, 20676, 20757 and 20797 were used before and after variation.

The staphylococci were cultured on nutrient agar and incubated aerobically for 18 hours at 37°C. Cells were suspended in saline, the concentration of viable cells per ml being between about 1 and 3 x 10⁹ viable cells.

White rabbits were intradermally inoculated with 0.1 ml of the above suspensions or with 0.1 ml of supernatent liquid of staphylococci grown for 3 days in Todd-Hewitt broth in an atmosphere containing 30% CO₂. The presence of staphylococcal toxin for rabbits was also tested for by intraperitoneal injection of supernatant into mice and by the demonstration of hemolytic activity on a 2% v/v suspension of rabbit erythrocytes.

**Results**

**Intracerebral inoculation in mice.** Table 1 shows the results obtained after intracerebral inoculation of three *Staphylococcus* strains, two having antigen 17 and the third, antigen 13. In all three cases the same strain was inoculated before and after antigen loss variation.

Five hundred seventy-six mice were utilized in all. The results were statistically significant and it may be said that in the chosen experimental conditions, the strains with antigen 17 proved significantly more virulent for the mouse than after they have lost antigen 17 and acquired antigen 1.

**Intraperitoneal inoculation in the mouse.** For intraperitoneal inoculation in the mouse to be lethal, a comparatively
### TABLE 1.

**ANTIGEN LOSS VARIATION AND VIRULENCE IN STAPHYLOCOCCUS AUREUS (intracerebral inoculation in mice)**

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>VARIATION 17 → 1</th>
<th>VARIATION 13 → 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Results after 48 H</td>
<td>Results after 48 H</td>
</tr>
<tr>
<td></td>
<td>Dead</td>
<td>Alive</td>
</tr>
<tr>
<td>17 Risk</td>
<td>102</td>
<td>48</td>
</tr>
<tr>
<td>1 Risk</td>
<td>7</td>
<td>76</td>
</tr>
<tr>
<td>110 Risk</td>
<td>126</td>
<td>112</td>
</tr>
</tbody>
</table>

χ² = 75.2, p < 0.01

### TABLE 2.

**INTRAPERITONEAL INFECTION IN MICE OF DIFFERENT CULTURES WITH ANTIGENS 17 OR 13. RESULTS AFTER 48 H.**

<table>
<thead>
<tr>
<th>ANTIGENS 17</th>
<th>DEAD</th>
<th>ALIVE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Streptococcus, S 74, S 87, S 249)</td>
<td>50</td>
<td>38</td>
<td>78</td>
</tr>
<tr>
<td>ANTIGENS 13</td>
<td>DEAD</td>
<td>ALIVE</td>
<td>TOTAL</td>
</tr>
<tr>
<td>(Streptococcus, S 74, S 87, S 249)</td>
<td>20</td>
<td>68</td>
<td>88</td>
</tr>
<tr>
<td>70</td>
<td>67</td>
<td>137</td>
<td></td>
</tr>
</tbody>
</table>

χ² = 54, p < 0.01

(Cumulative results of four experiments)
large inoculum, ranging between $0.5$ and $2 \times 10^9$ viable cells, is necessary. Table 2 shows the results obtained after the intraperitoneal inoculation of several strains of staphylococci having either antigen 17 or antigen 1.

The conclusion for the intraperitoneal method is thus the same as for intracerebral one, i.e., greater virulence of strains with antigen 17 compared with strains which had been transferred several times in the laboratory and which show only antigen 1.

**Demonstration of the staphylococcal toxin.** The mice intraperitoneally inoculated with the supernatant fluids of broth cultures of staphylococci had different fates, depending on whether the strain grown in a liquid medium had antigen 17 or antigen 1, as shown in Table 3.

Though the number of observations is small, the response seems to be very clear, there being a greater production of toxin in those strains having antigen 17.

The demonstration of alpha toxin in the supernatants was further investigated by intradermal injection into rabbits and by haemolysis of a 2% suspension of rabbit erythrocytes. In both cases, the results agreed with those obtained by intraperitoneal injection in the mouse. For example, the supernatant of a culture with antigen 17 (strain 20797) gave rise to necrotic reaction when injected intradermally which was still very much apparent with the supernatant diluted to 1:100, whereas the similarly diluted supernatant of a culture with antigen 1 (strain 20797) did not evince any action after intradermal injection in the same animal. Similarly, the first supernatant showed haemolytic activity after diluting down to 1:63, whereas the corresponding value for the supernatant of the culture with antigen 1 was about 1:12.

**Discussion**

The results obtained make it possible to state that most of the recently isolated bacteria having antigen 17 are more virulent for the mouse than those which have already lost this antigen and have acquired antigen 1. The first explanation would be to consider antigen 17 (also antigen 13) a phagocytosis-inhibiting antigen of virulence, rather like M antigen of Streptococcus, the carbohydrate capsule of Pneumococcus or even the cord factor of Mycobacterium.

In Table 4 we show in outline this interpretation of Staphylococcus virulence and infection.
TABLE 3.

INTRAPERITONEAL INJECTION IN MICE OF SUPERHSTASIS OF NERVE CULTURES OF STAPHYLOCOCCI. Results after 48 h.

<table>
<thead>
<tr>
<th></th>
<th>DEAD</th>
<th>ALIVE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0799</td>
<td>37</td>
<td>5</td>
<td>42</td>
</tr>
<tr>
<td>20179</td>
<td>9</td>
<td>19</td>
<td>28</td>
</tr>
</tbody>
</table>

$\chi^2 = 25.9 < 0.01$

(Cumulative results of four experiments)

TABLE 4.

STAPHYLOCOCCAL INFECTION AND DISEASE

VIRULENCE

Infectivity
- Complete
- Partial
- Noninfectious

Pathogenicity
- Toxic
- Hemolytic
- Leukocytolytic
- Exotoxigenic

STAPHYLOCOCCAL DISEASE

Resistance
- Non-specific resistance
- Specific resistance
- Antibodies
It would probably be necessary for the factors responsible for resistance to be weakened in order for the virulence factors (infectivity plus pathogenicity) to prevail and cause staphylococcal disease. We also believe that the capacity to produce the various factors of infectivity and of pathogenicity may vary greatly from strain to strain. However, even strains with such capacities developed to a high degree will not bring about the disease if the resistance mechanisms remain normal.

So far our phagocytosis experiments have not revealed indisputable phagocytosis-inhibiting activity of antigen 17 that would explain the spectacular difference in the response in the mouse to the intracerebral injection of antigen 17 and antigen 1. We believe, therefore, that other factors may also have an outstanding role here (for example the alpha toxin), and that the antigen loss variation may be simultaneously accompanied by loss or weakening of other biological characteristics. In the experiments we described we demonstrated such weakening in relation to alpha toxin. In fact, all the studied strains with antigen 17 or antigen 13, produced in vitro a much greater amount of alpha toxin than the same strains after antigen variation to strains with antigen 1 or antigen 3.

Several papers have recently appeared on the greater virulence of Staphylococcus strains as a result of surface antigens which retard or inhibit phagocytosis (Koenig 1962; Koenig and Melly 1965; Morse 1965; Mudd 1965; Kapral 1965). It should be stressed, however, that much research was done on rare special strains, a good instance of which are the two colonial variants of the Smith type. These are, therefore, conclusions based on exceptional strains, by contrast with the phenomenon we described which is general, and applicable to some 80% of coagulase-producing Staphylococcus aureus strains freshly isolated from pathological specimens or from carriers.

Particularly noteworthy is the work of Beining and Kennedy (1963). Although these investigators used only a single S. aureus strain grown in vivo in the guinea pig and in vitro in the laboratory the authors concluded that they differed significantly in eight characteristics such as the respiratory rate, the virulence test in the rabbit and in the mouse or the production of haemolysin. Although we did not try the strains of Beining and Kennedy, we believe that the particular case they describe falls within our general range of strains originally having antigen 17 and which had lost it
more or less easily in vitro in favour of antigen 1. Also,
we can give no other interpretation to the differences in
virulence described by Koenig and Melly (1965) for three
strains after cultivation in laboratory media. We believe
that these are clear examples of the association of antigenic
variation with loss of virulence.

Virulence for animals must run parallel to virulence for
man. We believe that in laboratory strains—in which the
loss of antigen 17 or antigen 13 is an indicator of "ageing"
or "biologic fatigue"—there is less production of various
virulence factors (infectivity and pathogenicity). Most stud-
ies on the virulence of staphylococci in which biological
modification (which we think inevitable) was not taken into
account are open to criticism.

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