MICROBIOLOGY AND IMMUNOLOGY

THE EXPERIMENTAL PRODUCTION OF VACCINE STRAINS
OF SHIGELLA FLEXNERI AND THE STUDY OF THE IMMUNOLOGICAL
EFFECTIVENESS OF A LIVING DYSENTERY VACCINE

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The problem of obtaining living vaccines for the specific prophylaxis of enteric infections continues to
be one of urgent importance. Great importance in this respect is attached to work on the development of a
living dysentery vaccine [16, 19].

In the present paper we describe the results of four years' (1954-57) experimental research on the pro-
duction of vaccine strains of Shigella flexneri and on the investigation of the properties of the living dysentery
vaccine [4, 5].

| Table 1 |
The Principal Biological Properties of the Test Strains

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Identification No. of strain of Shigella flexneri</th>
<th>Whence isolated</th>
<th>Date of isolation</th>
<th>Virulence LD50 (in 10³)</th>
<th>Antigenicity in %</th>
<th>Toxicity, LD50 (in 10³)</th>
<th>Titer of agglutination with specific serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No. 6267, type c</td>
<td>From stools</td>
<td>10/22/52</td>
<td>250</td>
<td>86</td>
<td>4</td>
<td>1:12800+++</td>
</tr>
<tr>
<td>2</td>
<td>No. 6211, type f</td>
<td></td>
<td>10/17/53</td>
<td>250</td>
<td>75</td>
<td>4</td>
<td>1:12800+++</td>
</tr>
<tr>
<td>3</td>
<td>No. 266, type a</td>
<td></td>
<td>2/15/50</td>
<td>250</td>
<td>80</td>
<td>2</td>
<td>1:12800+++</td>
</tr>
</tbody>
</table>

EXPERIMENTAL METHOD AND RESULTS

Three strains of Shigella flexneri—No. 6267 type c, No. 6211 type f and No. 266 type a—were used in
the experiments. The first two were obtained from the patients with acute dysentery and the last was a pro-
duction strain obtained from the government control institute. Our aim was to obtain a considerable and lasting
fall in the virulence of these strains while preserving their antigenicity and other biological properties.

For this purpose we carried out experiments on the cultivation of Shigella flexneri in bile in conjunction
with passage through cold-blooded animals insusceptible to dysentery. Bile is known to stimulate the growth of
a number of microorganisms and to stabilize their newly acquired properties. Repeated subculture in bile broth
TABLE 2. Characteristics of the Principal Properties after 10 Passages through the Frog's Brain

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Strain no.</th>
<th>Morphological and staining properties</th>
<th>Biochemical properties</th>
<th>Form of dissociation</th>
<th>Titre of agglutination</th>
<th>Virulence (in (10^9))</th>
<th>Antigenicity (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6267</td>
<td>Typical</td>
<td>Typical</td>
<td>S</td>
<td>1:12 800</td>
<td>5</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>6211</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1:12 800</td>
<td>1</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>266</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1:12 800</td>
<td>1</td>
<td>80</td>
</tr>
</tbody>
</table>

of the microorganisms of anthrax, swine erysipelas and other diseases brings about a sharp reduction in their virulence and stable preservation of newly acquired properties [17]. When *Shigella flexneri* is grown in 20% bile broth, a sharp decrease in its virulence is observed [10].

The use of cold-blooded animals for the experimental cultivation of dysentery microorganisms was established long ago. In 1884, I. I. Mechnikov observed that the virulence of *Bacillus anthracis* was greatly diminished by injection under the skin of frogs. When toads (*Bufo vulgaris*) were inoculated with *Bacillus pestis*, it was found [3] that the longer the organism existed in the body of the cold-blooded animals, the greater was the decrease in its virulence. Similar experiments with *Bacterium tularense* also confirmed the possibility of lowering the virulence of these microorganisms [12].

The dysentery strains tested all possessed characteristic biological signs (Table 1).

All the properties were assessed by the generally accepted method. The virulence and toxicity were estimated by the value of the \(LD_{50}\), the results obtained being treated by the method of Read and Mench.

It will be seen from Table 1 that the strains possessed high virulence and antigenicity and low toxicity and were agglutinated to a titre of 1:12 800. Also, they were present in the S-form, they possessed high specificity and were phagolytic.

In the first series of experiments the optimal concentration of bile-peptone broth and cultivation time were selected. As a result several important factors were elucidated. The greatest fall in virulence took place during cultivation at constant temperature (37°C) in 60% bile-peptone broth. From the \(LD_{50}\) values, the virulence of strain No. 6267 was 5 x \(10^9\)/ml, and that of strains Nos. 6211 and 266 was 750 x \(10^6\)/ml. The antigenicity remained high (between 60 and 80%), even after cultivation for 120 days in 60% bile-peptone broth. The remaining biological properties, namely agglutinability, specificity, form of dissociation, saccharolytic activity, etc, remained unchanged. It was remarkable that the cultivation of *Shigella flexneri* in bile-broth brought about a decrease in virulence but usually stabilized all the remaining properties typical of this species of microorganism.

However, the level to which the virulence of strains Nos. 6211 and 266 had been lowered was still too high, and furthermore, after passage through white mice, the virulence reappeared. We therefore decided to cultivate the microorganisms in bile-peptone broth and to combine this with cultivation in cold-blooded animals. These experiments were performed on pond frogs (*Rana radibunda*), which were inoculated intracerebrally. From our point of view, this method of introduction of the infective material had two advantages; firstly, the usual medium for its existence in an insusceptible animals must promote a further lowering of the virulence of the microorganisms, and secondly, it would be easier to obtain a pure culture, uncontaminated by other microorganisms, from the frog's brain.

The culture was injected into the midbrain in the region of the posterior third of the parietal bones. A 24-hour culture, suspended in physiological saline, containing \(10^9\) bacterial cells per ml was used for inoculation. The volume of the suspension injected was 0.05 ml. For passage, 48 hours after inoculation, the frog's skull was opened, the brain extracted and seeded with a pure dysentery culture which, after thorough testing, was again injected into the brain of a frog. Ten passages were performed.

Table 2 shows the results of tests of the strains after the last passage, indicating that they preserved their