INCREASED VIRULENCE OF THE HOG-CHOLERA BACIL-
LUS PRODUCED BY PASSAGE THROUGH RABBITS.

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In a previous paper (1) the writer mentioned the attempt to modify the carbo-
hydrate reactions of a culture of the hog-cholera bacillus by passing it through
a series of rabbits. The only change noted was an increase in virulence, and it
is the object of this paper to record this change.

Since the time of Pasteur many organisms have had their virulence increased
by animal passages but the recorded results of the passage of the hog-cholera
bacillus through rabbits are somewhat conflicting. Moore (2) passed a typical
organism through a series of twenty-six rabbits and concluded that there was no
increase in virulence for this animal. He judged the virulence by the time taken
to kill the animal and did not consider the amount of culture necessary to produce
death. Previous to this Selander (3) had reported the rapid increase in virulence
of a hog-cholera bacillus by rabbit passages and his work was apparently confirmed
by Metchnikoff (4). Smith and Moore (5) had shown, however, that Metchni-
koff was working with the swine-plague bacillus and as Moore could not confirm
the work of Selander he concluded that the latter was probably also working
with the swine-plague organism.

Smith (6) later worked with a culture of the hog-cholera bacillus of low initial
virulence further attenuated by age, which would not kill rabbits when injected
under the skin. By animal passages, he increased the virulence to the point
where subcutaneous injections were fatal.

Later Smith and Reagh (7) in attempting to modify the agglutinability of a
strain of the hog-cholera bacillus, passed it through a series of fourteen rabbits.
As a result of this passage the virulence was increased from a minimal fatal dose
of 0.1 cc. in the stock strain to 0.00001 cc. in the passage strain. It has already
been pointed out (1) that this increased virulence still persists to a certain degree
after a lapse of 15 years.

The organism with which we worked is a stock culture known as
Hog-cholera XII. It was isolated by Dr. Smith in 1914 from the
spleen of a pig dying from hog-cholera. Soon after this it was passed
through a rabbit and since that time it has been kept on slant agar
in the cold, transfers being made monthly.
Culturally it is a motile, Gram-negative rod, growing readily on the ordinary media and forming acid and gas in dextrose bouillon but not attacking lactose or saccharose. It is quantitatively agglutinated by serum from rabbits injected with other strains of the hog-cholera bacillus and when injected into animals it causes the production of agglutinins for other strains of the hog-cholera bacillus.

Starting in January 1916 this culture was passed through a series of eleven rabbits; the essential details of this passage will be found in Table I. The passage was made directly from one animal to the next by using a suspension of crushed spleen for the inoculation. After the third transfer the inoculation was made by rubbing a small amount of the spleen suspension into the shaven skin of the next rabbit.

TABLE I.

Passage of Hog-Cholera XII through Rabbits.

<table>
<thead>
<tr>
<th>Passage</th>
<th>Weight of rabbit</th>
<th>Method of infection</th>
<th>Material and dose</th>
<th>Length of life</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,199</td>
<td>Subcutaneous</td>
<td>0.5 cc. of 24 hr. bouillon culture.</td>
<td>6</td>
<td>Typical lesions.</td>
</tr>
<tr>
<td>2</td>
<td>1,172</td>
<td>&quot;</td>
<td>Spleen suspension.</td>
<td>6</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>3</td>
<td>1,274</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>7</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>4</td>
<td>1,305</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>7</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>5</td>
<td>1,430</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>7</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>6</td>
<td>2,073</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>9</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>7</td>
<td>2,089</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>9</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>8</td>
<td>2,273</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>8</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>9</td>
<td>2,029</td>
<td>&quot;</td>
<td>&quot; &quot;</td>
<td>7</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>10</td>
<td>2,306</td>
<td>Subcutaneous</td>
<td>0.000001 cc. of 24 hr. bouillon culture from Rabbit 9.</td>
<td>9</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>11</td>
<td>2,330</td>
<td>&quot;</td>
<td>0.00000001 cc. of 24 hr. bouillon culture from Rabbit 10.</td>
<td>6</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

It would be difficult to estimate accurately the relative virulence of this culture from the duration of the disease in the various animals but a comparison of the number of organisms necessary to kill gives us very definite results. With the stock culture one must use somewhat over 0.00001 cc. of a 24 hour bouillon culture to produce death.
Of four rabbits given a subcutaneous injection of this amount one died in 12 days, one showed an increased temperature and loss in weight, the other two showed no effects. One rabbit that was given 0.001 cc. subcutaneously died in 4 days. With the strain of the same bacillus passed through the rabbits, 0.00000001 cc. of a 24 hour bouillon culture injected subcutaneously into a rabbit weighing 2 kilos causes death in about 6 days. The passage strain has therefore been increased in virulence about one thousand times and plate counts show that the number of organisms necessary to infect has been reduced from approximately 20,000 to 20.

The type of disease produced by the more virulent organism shows no striking departure from that caused by the original culture. This organism seems to have a greater power of penetration than do most cultures of the hog-cholera bacillus, as it causes only a slight local lesion. The bacteria apparently enter the body through the lymphatics, for the axillary and inguinal lymph nodes on the side of the inoculation are enlarged and congested and often show large areas of necrosis.

The passage through rabbits has produced no change in the morphology of the organism that can be detected either in films made from the spleen of an animal dying from infection or in films made from cultures. The passage strain is slightly more motile than the original culture but this difference is not marked. It is also more susceptible to agglutinins, in that clumps are formed in a shorter time, but the readings after 2 hours’ incubation are the same. The passage of the original strain through one rabbit makes it as susceptible to agglutinins as is the strain passed through eleven rabbits. With some immune sera the parent strain is agglutinated in slightly higher dilutions than is the passage strain but here too the difference is not great.

SUMMARY AND CONCLUSION.

By passage through a series of eleven rabbits a culture of the hog-cholera bacillus has increased its virulence a thousand times. A subcutaneous injection of twenty organisms, or 0.00000001 cc. of a 24 hour bouillon culture, or a drop of a bouillon culture rubbed lightly into the shaven skin, produces, in the rabbit, a characteristic disease resulting in death on or about the 6th day.
VIRULENCE OF THE HOG-CHOLERA BACILLUS

BIBLIOGRAPHY.