PROTECTION AGAINST TYPHOID-LIKE INFECTIONS BY VACCINATION.

AN EXPERIMENTAL STUDY.

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The basic fact that infections with the typhoid bacillus can be prevented by vaccination is, of course, well established by clinical evidence. It is also supported, theoretically, by a certain amount of experimental evidence. Problems still remain, however, in regard to the degree and duration of immunity, the best kind of vaccine, its keeping qualities, and the best method of administration. The experimental solution of these problems has been delayed by the impossibility of reproducing a true typhoid infection in the common laboratory animals. In the absence of a real protection test, efforts have been made to settle many questions by a quantitative determination of the antibody content of the serum of vaccinated animals. This method has been of practical value in some directions, but of course, in general, is inferior to an actual protection test. As an illustration of the limitations of the antibody method, it may be stated that typhoid vaccine, 10 years old, produces almost as good an agglutination titer in the rabbit as fresh vaccine, but its protective value is much less.

Another method of testing immunity has been the use of typhoid intoxication in the common laboratory animals. By intravenous or intraperitoneal injections of living bacilli, differences in toxicity can be determined in different strains of the typhoid bacillus and protection by vaccination can be demonstrated. This method also has some value, but it is highly artificial, as the doses are usually enormous and
there is no true septicemia. The same is true of the Gay method of using gall bladder infections as a test of immunity.

In recent years the discovery of the natural occurrence of paratyphoid infections in animals as well as in man has opened the way for actual protection tests with this group of organisms. Clinically, paratyphoid vaccination is as effective as typhoid vaccination and there seems to be no reason why the information obtained from vaccination with the paratyphoid group of bacilli should not be applied to the whole Salmonella group. This method has recently been used, experimentally, by Besredka (1) in developing his ideas on local immunity and by Flexner and Amoss (2), Webster (3), and Lynch (4) in epidemiological work among mice. We have also used this method for several years in attempting to solve some of the problems mentioned above, in connection with the manufacture of large amounts of triple typhoid vaccine at the Army Medical School. Even this method, in our hands, is somewhat artificial, but the results are none the less definite and valuable.

Considerable work along the same lines has been done with other groups of organisms, especially the Pasteurella group. The most extensive recent work has been that of Harvey (5), who worked with pigeons and after vaccination with *B. avisepticus*, gave intravenous infecting doses. He concludes (a) that an avirulent organism protects as well as a virulent one, (b) that multiple small doses protect better than one large dose, (c) that there is some group and non-specific protection, and (d) that vaccine does not deteriorate with age or temperature up to 6 to 9 months.

Our work started in 1921 with a spontaneous and severe epidemic among guinea pigs, which furnished us with a picture of the natural disease. The causative organism was the "mutton" strain of *Bacillus aertrycke*, which can be differentiated from the human paratyphoid B bacillus only by absorption of agglutinins.

*Method.*

Male guinea pigs of approximately the same weight were selected and kept under observation for about a week. They were then vaccinated, subcutaneously, at 8 to 10 day intervals and 15 days after the last dose of vaccine were given the infecting dose, also subcutaneously.

This dose was about 400 million organisms. The virulence was such that the controls died in 6 to 10 days. Each animal that died was autopsied and the lesions were noted and cultures were made from the spleen, liver, gall bladder, and heart's blood. Surviving animals were chloroformed and autopsied after 30
days and similar cultures were made. The organisms were identified by agglutination. A few surviving animals showed possible residua of infection with negative cultures, and were considered not infected. Precautions were taken to avoid sudden drop in animal room temperature which is often followed by a number of deaths from non-specific pneumonia.

Careful adjustment of the virulence of the virus is necessary and several preliminary series were lost through failure to observe this point. In one series, all the vaccinated animals died as well as all the controls, although the vaccinated animals lived longer and had less extensive lesions. In another series, none of the controls became infected. It was finally found that by making blood cultures from sick animals and by transplanting to blood broth, the virulence could be maintained at a fairly fixed point. The infecting doses were made from 18 hour blood broth cultures. In each experiment, as far as possible, four animals were used, with four normal controls, and four controls vaccinated with fresh, full strength, saline vaccine, 1 cc. of which contained about 1,000 million organisms.

An attempt was made to imitate natural conditions by feeding bacilli and by inoculation of the alimentary tract by means of a medicine dropper and fairly good results were obtained in one series, but this method was given up for the subcutaneous one, on account of more certainty of infection.

The animals all developed a local lesion at the site of inoculation consisting of a suppurating nodule which usually broke down and discharged. In normal animals this lesion was large and edematous; the animals appeared sick and usually had a conjunctivitis. In protected animals, the lesion was sharply localized and no systemic reaction was noted. The specific internal lesions in non-protected animals consist of an enlarged and granular spleen and liver, an infected gall bladder, and usually a pneumonia.1

Control Experiments with Full Strength Fresh Saline Vaccine.

TABLE I.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Protected</th>
<th>Not protected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Vaccinated (2,500 million)</td>
<td>11</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>

These results were all clean-cut; the controls all died of the acute disease and all cultures were positive except in one instance of death on

1 The experimental stock was obtained by courtesy of the United States Department of Agriculture and was free from natural infection.
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the 27th day in which the organisms were found only in the gall bladder contents (Table I). None of the vaccinated animals showed any signs of disease except locally. The vaccinating doses were 500, 1,000, and 1,000 million bacilli subcutaneously at 8 day intervals, a total of 2,500 million organisms. This technique affords, therefore, a definite method by which it is possible to secure reliable data on various questions.

Relation of Dose of Vaccine to Protection.

The doses used to vaccinate animals are usually the same as for adult human beings and of course are enormously larger than comparative body weight would call for. The infecting dose, however, is also much larger than the proportionate dose for man. But the effect of different sized doses of vaccine is instructive.

<table>
<thead>
<tr>
<th>Total No. of organisms injected</th>
<th>No. of animals</th>
<th>Protected</th>
<th>Not protected</th>
</tr>
</thead>
<tbody>
<tr>
<td>625 million.</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>300 &quot;</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>25 &quot;</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

The vaccine was given in the regular way in three doses of 0.5, 1.0, and 1.0 cc. The dose in the third experiment, is the adult human dose reduced approximately to body weight for the guinea pig. Only one out of three animals was protected (Table II). All the animals in this series, which are marked not protected, died of the disease, with positive cultures. It is seen again that the protection varies with the number of organisms injected.

Effect of Age of Vaccine on Protection.

The question of the keeping power of prophylactic vaccines is of importance, economically, as well as immunologically. Attempts to settle this question by antibody production are not convincing. As will be seen from Table III, there is a falling off of protection after 8 months and we believe that this is about the time limit for this kind of vaccine.
TABLE III.

<table>
<thead>
<tr>
<th>Age of vaccine</th>
<th>No. of animals</th>
<th>Protected</th>
<th>Not protected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>11</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

All the animals marked not protected died of the disease, except one of the 11 months series, which survived but on autopsy showed lesions, with organisms, in the spleen and gall bladder. The vaccine was kept under conditions which resembled those of actual use, part of the time at room temperature and part of the time in the ice box, with occasional shaking. It is seen that vaccine up to 8 months old gave complete protection in twenty-two animals. Vaccine 10 to 14 months old protected only eight out of twelve animals, a falling off of one-third in protective power.

**Effect of Different Kinds of Vaccine.**

The exact kind of vaccine has, of course, been the subject of much work and discussion. Our results with several different kinds are given in Table IV.

TABLE IV.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>No. of animals</th>
<th>Protected</th>
<th>Not protected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipovaccine (2,500 million)</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Resuspended vaccine</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Supernatant fluid</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sensitized vaccine (killed)</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>T. A. B. vaccine</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

*Lipovaccine.*—The animals were given a single dose in oil equal to the divided saline doses. All the animals survived, but two on autopsy showed specific lesions of the spleen and gall bladder with positive cultures.
Resuspended Vaccine.—The vaccine was allowed to stand for about 14 days in 5 cc. ampules until the supernatant fluid was crystal-clear. This fluid was pipetted off and the sediment was resuspended in an equal amount of salt solution. The results were as good as with the original vaccine.

Supernatant Fluid.—The supernatant fluid, obtained as stated above, was used as a vaccine. This fraction has some protective power, but one animal died of the disease and one of three surviving animals was infected.

Sensitized Killed Vaccine.—This was apparently not so effective as the original vaccine, as one animal died of typhoid disease. Four animals, given a living sensitized vaccine, all died of the disease contracted from the first dose of vaccine.

T. A. B. Vaccine.—This group vaccine protected fully. The total Para B dose was 1,250 million, Para A, 1,250 million, and typhoid, 2,500 million.

We also have some partial data which tend to show that detoxicated (6) and filtered vaccines do not protect so well as the original vaccine. Our work on vaccination by mouth (Besredka) is too incomplete to be discussed, but Webster (3), working with the same organism in mice, found no evidence of local immunity.

DISCUSSION.

The experimental work cited above supports the following conclusions and general rules for practise in prophylactic vaccination against the typhoid group of infections. It may appear that some of this work is superfluous, as several of the conclusions reached are already accepted, but it seems desirable to record all the actual evidence obtainable.

1. Acquired immunity is a variable factor and depends partly on the dose of vaccine. A high immunity can be obtained by a sufficient vaccination but can be overcome by a large enough infecting dose. Repeated doses give better immunity than a single dose. It is probably also true that frequent courses give the greatest protection, which is probably the reason for the freedom of the Army from these infections. According to regulations, vaccination is given every 3 years for three courses. But not infrequently records are lost and vaccinations are repeated oftener than called for.

2. A relatively fresh vaccine gives better protection than an old one; the period of maximum efficiency is about the first 8 months.

3. Plain saline vaccine is superior to lipovaccine, sensitized vaccine, or some fractions. Vaccination with sensitized living vaccine produced the disease instead of protecting.
The principal point of interest in this connection is probably in regard to the use of lipovaccine. It is not intended to discuss the subject fully here, but it may be said that the use of lipovaccine was discontinued in the Army, primarily, on account of the difficulty of making a sterile product on a large scale. When methods were available for making a sterile vaccine, lipovaccine was not reissued because of doubts as to its protective power. As judged by the production of antibodies, it is distinctly inferior to saline vaccine (7). Judged by the present method it undoubtedly gives some protection and in an emergency might be used on account of the definite advantages of a single dose. The best protection, however, is afforded by multiple doses of a saline vaccine.

The subject of group and non-specific immunity has come to the front recently. A partial non-specific protection has been demonstrated experimentally by Harvey (5), by Clark (8), and by others. According to the results given above, a group vaccine proved as effective as the specific vaccine. It should be remembered, however, that the human Para B bacillus is very closely related to Bacillus aertrycke. In our Service, on the Mexican Border before the War, typhoid vaccine, alone, gave no adequate protection against infections with Para A and B. During the war, the same fact was demonstrated in the British Army and a paratyphoid vaccine was insisted on and proved effective.

SUMMARY.

1. A natural infection of guinea pigs with the "mutton" strain of Bacillus aertrycke was used to test the protective power of vaccination against the typhoid group of infections.
2. Under the conditions of the experiment, complete protection was secured by vaccination with full strength fresh saline vaccine, while 100 per cent of deaths occurred among the controls.
3. The immunity acquired is variable and depends on the number of organisms injected.
4. Vaccine kept 10 to 14 months gave less protection than vaccine 8 months old and under.
5. Saline vaccine was more effective than lipovaccine, sensitized vaccine, or supernatant fluid vaccine.
6. Resuspended vaccine was as effective as the original vaccine.
7. In one experiment, group vaccine, made of typhoid Para A and Para B bacilli, was as effective as the original specific vaccine.

BIBLIOGRAPHY.