Preliminary Experiments with a View to the Preparation of a Non-Toxic Dyentery Vaccine.

By Major F. R. Copinger,
Royal Army Medical Corps,

and

Captain R. C. Robertson,
(Late) Royal Army Medical Corps
(Pathological Department, Royal Army Medical College).

A comparison between the incidence of the enteric group of infections and bacillary dysentery during the recent war evidences in a striking manner the effects of prophylactic vaccination against the former group of diseases. The occurrence of enteric infections almost disappeared, and the severity of those cases which did occur was much diminished. On the other hand, bacillary dysentery was widespread on every front, and in the closing years of the campaign constituted a serious cause of wastage of man power. In certain instances attempts were made to immunize small numbers of men by means of prophylactic inoculation, but very little success attended any of the measures employed.

This failure to produce immunity may have been in part due to the fact that bacillary dysentery is not, as a rule, associated with a general blood infection, as in the case of the enteric group of diseases, but the chief difficulty was the impossibility of inoculating sufficiently large doses of vaccine owing to the severity of the reactions produced.

It is evident, therefore, that if any method can be devised which will diminish the toxic properties of the bacillus without affecting its antigenic
The Preparation of a Non-toxic Dysentery Vaccine

The preparation of a non-toxic dysentery vaccine would make considerable advance in the possibilities of vaccine prophylaxis. Various methods have been suggested towards this end, and of these sensitized and sero-vaccines have probably found most favor.

**Previous Methods of Preparing Vaccines for Bacillary Dysentery.**

*Sensitized Vaccines.*—Sensitized vaccines, prepared in the ordinary manner by treating suspensions of the bacilli with their homologous sera, and, after incubating for some time, removing the serum by means of the centrifuge, have been employed to a considerable extent, but not with much success, since they remained toxic and produced severe reactions.

*Sero-vaccines.*—The Japanese method consisted in injecting an equal bulk of anti-serum at the same time as an ordinary vaccine prepared from dysentery bacilli. The toxic effects in this case were neutralized, but it appeared that the antigenic properties were also greatly diminished.

Gibson [1] working on the same lines produced a sero-vaccine composed of a mixture of anti-serum with ordinary dysentery vaccine, but he attempted to remove the anti-bacterial substance from the serum prior to injection, while leaving the anti-toxic substance unaffected. His method for removing the anti-bacterial substance was to absorb the serum with dysentery bacilli, and then remove the organisms by passing the treated serum through a filter candle. As far as can be ascertained, however, from a limited trial of this vaccine the immunity produced was not sufficient to warrant its use on a large scale.

*Lipo-vaccines.*—Lipo-vaccines, which consist of a suspension of the organisms in some oily substance, have also been employed in prophylactic inoculation for dysentery; the basic principle underlying this method being that the oily substance delays absorption and thus prevents the injurious effects of the toxins on the tissues. Early in 1918 Whitmore and Fennel [2] of the U.S. Army prepared a lipo-vaccine for dysentery on a large scale by growing the bacteria in Kolle flasks, removing the growth with a vacuum scraper, freezing and drying in vacuo, and emulsifying in lanolin and oil by grinding in a ball mill. The oils were sterilized by steam at fifteen pounds for fifteen minutes, by heating to 90° C. for ten hours in a water bath or by mixing with potassium iodide. They gave very large doses, up to 3,000 million Shiga, 3,200 million "Y," and 2,200 million Flexner, without marked local or general reaction, and claimed to have demonstrated the presence of agglutinins, precipitins, and bacteriolysins in the blood of the inoculated animals and men. The difficulty, however, of preparation, especially in the sterilization of the oils, was so great that this vaccine was abandoned by the U.S. Army shortly after the armistice.

*Chemically-treated Vaccines.*—Chemically-treated vaccines are a comparatively new departure in vaccine therapy, and, judging by the results
already claimed by various authors, there appears to be every prospect of their being used with success in the future.

Very little work, however, has been carried out along these lines in the case of dysentery bacilli, although they form one of the most important groups for which a non-toxic antigen is required.

In 1916, Dean and Adamson [3] found that by suspending Shiga bacilli in dilute eusol solution, the toxicity of the organism was reduced, but subsequent experience proved that the irritant effects of the inoculation were not eliminated to a degree which would permit of its general use as a vaccine. Especially was this the case when the vaccine was stored for some time after preparation.

More recently Jötten [4] claims to have produced a non-toxic antigen for Shiga bacilli by treating a suspension of the organisms with dilute antiformin and subsequently neutralizing and dechlorinating the mixture. It was found, however, on repeating his experiments, that although the toxicity was undoubtedly diminished, it was not reduced to the degree claimed in his paper. It is possible, of course, that this discrepancy in findings may have been partially due to a difference in toxicity of the strains of organisms used in each case.

As far as we are aware the above have been the only attempts at reducing the toxicity of Bacillus shiga by chemical means, and it appeared desirable to carry out further investigations on these lines.

The results claimed by Thomson [5] in the "detoxication" of various organisms by treatment with alkali suggested the application of a somewhat similar procedure in relation to B. shiga. Thomson found that many organisms rapidly dissolved in the presence of N/10 or N/20 sodium hydroxide, while others were comparatively insoluble even in much stronger solutions of the alkali. Many of the latter, however, showed evidence of solution in 10 per cent. NaOH, and all dissolved under prolonged treatment with antiformin at 37°C. If these alkaline solutions of bacteria were now neutralized with an excess of acid, a precipitate formed which, on being separated and washed, proved to be non-toxic, while the neutralized supernatant fluid remained toxic and caused similar reactions to that following inoculation of the untreated bacilli. It was further claimed that the non-toxic precipitate retained the antigenic properties of the organism, and produced on inoculation into animals a definite immunity without giving rise to the usual local and general reactions. Much larger doses could, therefore, be tolerated than in the case of the ordinary vaccine, and it was claimed that the immunity produced was proportionally much increased.

This, in short, was the theory of Thomson's original "detoxicated" vaccine, and although he has recently further developed the principle of splitting up the organism into its toxic and antigenic portions, the above remains the basis of all his subsequent work.
The Preparation of a Non-toxic Dysentery Vaccine

The experiments described in this paper refer only to the Shiga bacillus, but as this organism is by far the most toxic of the dysentery group, it is considered that, if its toxicity can be reduced without affecting its antigenic properties, little difficulty would be experienced with the other organisms of the group.

Preliminary attempts to dissolve suspensions of Shiga bacilli with decinormal and normal sodium hydroxide were not successful, and even stronger solutions up to about twenty per cent failed to break up the organisms. It was noted, however, that after contact with normal sodium hydroxide for one or two hours at 37° C. the bacilli appeared swollen and distorted in shape.

When a suspension thus treated was neutralized with normal hydrochloric acid it became more opaque, presumably owing to the formation of a very fine precipitate or colloidal suspension of some dissolved constituent of the bacilli. It was found, however, impossible to obtain any quantity of the residue on centrifugalization, the bulk of the swollen bacilli even remaining in suspension.

Attempts were now made by various methods to obtain this residue and it was found that the suspension, if treated with an excess of absolute alcohol, yielded a light flocculent precipitate which gradually subsided to the bottom of the flask, and could more rapidly be brought down by means of a centrifuge. This precipitate, on being washed and dried, formed a white powder, which on microscopical examination appeared to be formed of partially broken up and macerated bacilli.

Portions of this powder equivalent in weight to 20 million, 200 million, and 400 million unaltered Shiga bacilli were injected intravenously into rabbits. No lethal effects were produced and toxic symptoms did not appear. A low agglutination titre (1/100) was obtained in one animal after a course of three inoculations of about the equivalent in weight to 200 million untreated Shiga bacilli given at weekly intervals. This animal was then found to be protected against an inoculation of 200 million untreated bacilli, which, as will be seen later, represents approximately ten times the fatal dose for an animal of the same weight.

Steps were now taken to ascertain the minimum lethal dose of the strain of Shiga bacilli used in all these experiments so as to be able to form a more accurate estimation of the reduction in toxicity caused by the action of the alkali. A suspension of the bacilli was accordingly dried in vacuo, and the residue ground to a fine powder. Various quantities of this powder suspended in normal saline solution were injected intravenously into rabbits of approximately the same weight, and it was found that 0·01 of a milligramme (equivalent to about twenty million bacilli) was just sufficient to kill a rabbit weighing 1,000 grammes in three days. This quantity was accordingly counted as the minimum lethal dose, though it was found later...
that rabbits vary very considerably in their resistance, and that occasionally they could survive a slightly larger dose. It was never found, however, that even a large rabbit could resist more than 0.05 of a milligramme of dried Shiga bacilli injected intravenously unless it had been immunized by previous inoculation.

Similarly it was found that one milligramme of dried Shiga bacilli injected subcutaneously was just sufficient to kill a rabbit weighing 1,150 grammes in three days. This large dose was, of course, necessary owing to the slow absorption from the subcutaneous tissues.

In these experiments it was found that the rabbits died with paralytic symptoms, and practically no diarrhoea, and no post-mortem lesions of the intestines beyond a slight congestion could be demonstrated. Intestinal lesions, however, did occur in cases of delayed death following partial immunization.

The above method for reducing the toxicity of Shiga bacilli by treating them with NaOH, neutralizing with HCl, and precipitating with alcohol, appeared fairly satisfactory. The amount of alcohol, however, required for the precipitation was very considerable and it was therefore considered desirable to try some more economical method of preparation.

On attempting to precipitate with ammonium sulphate it was found that almost complete saturation produced a similar reaction to that of the alcohol, the precipitate, however, being heavier and sedimentation more rapid.

In order, therefore, to treat the organism with alkali and produce a strong solution of ammonium sulphate on neutralization, a new method was adopted. To a thick emulsion of Shiga bacilli an equal bulk of liquor ammonii fortis (B.P.) was added. The mixture was allowed to stand in the incubator at 37°C. over night, and neutralization was then effected by means of twenty per cent. sulphuric acid, which, combining with the ammonia, formed ammonium sulphate. No precipitation occurred until the neutral point was reached, when a heavy precipitate immediately formed which rapidly subsided to the bottom of the flask leaving a clear supernatant fluid.

On separating and washing the precipitate it was found to be composed of bacilli which were somewhat swollen and altered in shape, but still quite discrete and distinct.

Inoculation into rabbits showed that these altered bacilli were relatively non-toxic, but retained their antigenic properties.

A series of experiments was now commenced with a view to testing the antigenic power of the altered bacilli and determining the most suitable doses for immunizing purposes. The immunizing effects were estimated by injecting toxic Shiga bacilli into rabbits previously inoculated with the new antigen, and judging from the results the degree of protection which had been conferred. In the absence of symptoms the condition of the rabbits was judged by the weight, an accurate daily chart of which was kept in every case.
The Preparation of a Non-toxic Dysentery Vaccine

Little reliance was placed on agglutinin production in animals thus immunized, as it was not considered to give a true estimate of the degree of protection.

The immunizing doses were estimated by opacity, using Brown's barium sulphate tubes. In this connexion it may be noted that before making up the final emulsion, the altered bacilli were not, as a rule, completely dried, as it was found difficult to produce an even emulsion from the dry powder. A table was, however, prepared, showing the weight of dried antigen corresponding to each opacity tube.

A series of ten rabbits was inoculated, five subcutaneously and five intravenously, with ammonia-treated bacilli. The doses given and the results obtained are shown in Table I.

<table>
<thead>
<tr>
<th>Rabbits inoculated</th>
<th>Doses of treated Shiga bacilli, in milligrammes, at seven-day intervals</th>
<th>Agglutination titre of serum 24th day</th>
<th>Lethal doses of toxic Shiga bacilli 24th day, intravenously</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subcutaneously</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 346</td>
<td>0·5 1·0 5·0 1 in 120 10 M.L.D.</td>
<td>Survived. No symptoms beyond a slight temporary loss in weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 347</td>
<td>0·5 1·0 5·0 1 in 30 20 M.L.D.</td>
<td>''</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 348</td>
<td>1·0 2·0 7·5 1·0 1 in 120 10 M.L.D.</td>
<td>''</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 349</td>
<td>1·0 2·5 10·0 1·0 1 in 60 20 M.L.D.</td>
<td>''</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 350</td>
<td>2·0 5·0 10·0 1·0 1 in 130 20 M.L.D.</td>
<td>''</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intravenously</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 351</td>
<td>0·1 0·2 0·5 1 in 500 10 M.L.D.</td>
<td>Survived. No symptoms nor loss in weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 352</td>
<td>0·1 0·5 1·0 1 in 1,000 20 M.L.D.</td>
<td>Died fifth day after first inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 353</td>
<td>0·25</td>
<td>Died third day after first inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 354</td>
<td>0·5</td>
<td>Died second day after first inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 355</td>
<td>1·0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All the rabbits inoculated subcutaneously survived, and suffered from no obvious reactions, either local or general, though in each case a distinct drop in weight occurred for a few days after the lethal test dose, which was subsequently injected intravenously.

Three of the series injected intravenously died after the first inoculation which was apparently too large. But the two which had received 0·1 of a milligramme survived, and afterwards received larger doses, without showing any ill-effects. Moreover, the protection conferred was greater than in the case of the subcutaneously-injected rabbits, as was judged by the fact that the injection of the lethal test dose produced no loss in weight.

Antitoxic Power of the Serum of Immunized Animals.—An attempt was now made to test the antitoxic effect of serum obtained from the seven
rabbits surviving from the above experiments. Serum obtained from each of the rabbits was pooled, and one cubic centimetre of the pooled serum injected into each of three normal rabbits along with 0·8, 0·4, and 0·1 of a milligramme of unaltered Shiga bacilli respectively. The first two rabbits died, but the animal which had received 0·1 of a milligramme (10 M.L.D.) survived, showing that the serum had acquired some degree of antitoxic power. Table II shows the result of this experiment.

**TABLE II.**

<table>
<thead>
<tr>
<th>Source of serum employed (Control)</th>
<th>Amount of serum mixed with the toxin</th>
<th>Weight of dried toxic Shiga bacilli injected</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rabbit</td>
<td>nil</td>
<td>0·1 mgrm.</td>
<td>Died 2nd day</td>
</tr>
<tr>
<td>Inoculated rabbits (serum pooled)</td>
<td>1 c.c.</td>
<td>0·8</td>
<td>&quot; 3rd &quot;</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0·4</td>
<td>&quot; next &quot;</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0·1</td>
<td>Survived</td>
</tr>
</tbody>
</table>

**Human Experiments.**—Human experiments on any large scale have not yet been carried out, but one of us received injections of 0·03, 0·075, 0·15, and 0·3 of a milligramme of treated Shiga bacilli (approximately equal to 60, 150, 300, and 600 millions) without any reaction beyond a slight local tenderness and induration, which, in the case of the largest dose, persisted some days.

**Conclusions.**

The experiments described appear to prove that it is possible to reduce the toxicity of Shiga bacilli by chemical means without seriously reducing their antigenic properties. In the method employed this chemical action does not involve the complete breaking down and solution of the bacilli as in Thomson's detoxicated vaccines.

It is difficult to determine the exact action of the alkali on the organism, whether it removes some toxic constituent from the bacilli, or else changes the composition of the toxin, possibly reducing it to the toxoid state. The latter appears somewhat more probable as the supernatant fluid remaining after the precipitation of the bacilli was found to be non-toxic to animals.

Whatever may be the explanation of the action of the alkali, it would appear to be evident that the bacilli treated by this method are relatively non-toxic and may safely be used for commencing immunization in animals. The importance of this fact is obvious when it is remembered that there has always been a considerable mortality among laboratory animals during the early stages of immunization with unaltered Shiga bacilli.

Whether vaccines prepared on the same lines would be satisfactory for the prophylactic or therapeutic treatment of bacillary dysentery in human beings has yet to be proved.
250  The Preparation of a Non-toxic Dysentery Vaccine

Investigations on these lines are still in progress.

In conclusion, we wish to express our very grateful thanks to Colonel S. L. Cummins, C.B., C.M.G., A.M.S., Lieutenant-Colonel H. Marrian Perry, O.B.E., R.A.M.C., and the other members of the Advisory Committee on Pathology for their very valuable assistance and advice.

REFERENCES.

Preliminary Experiments with a View to the Preparation of a Non-Toxic Dysentery Vaccine
F. R. Coppinger and R.C. Robertson

J R Army Med Corps 1922 38: 243-250
doi: 10.1136/jramc-38-04-01

Updated information and services can be found at:
http://jramc.bmj.com/content/38/4/243.citation

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/