NOTES ON THE ROUGH AND SMOOTH FORMS OF BACILLUS TYPHOSUS.

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The controversy with regard to the theories of immunity in general, and Arkwright's work on the antigenic properties of "rough" and "smooth" colonies in particular, have naturally led those interested in the making of the Army vaccines to turn a critical eye on the components of their vaccines, as viewed in the light of the work of these experimentalists and the theories they have formed therefrom.

Many readers will be quite conversant with the points at issue, but, for the benefit of those who have not had the opportunity of seeing the various articles on the subject, I shall try to enumerate very shortly the conclusions arrived at by two workers which bear particularly on the subject of vaccine antigens.

1. — Felix, working with the sera of typhoid cases, described two forms of agglutination:
   (1) Floccular agglutination.
   (2) Granular agglutination.

   The floccular agglutination he associated with a heat-labile antigen and its corresponding agglutinin antibody, and found it to be closely connected with the flagella of the organism.

   The granular agglutination Felix associated with a heat-stable antigen.
and its antibody, and this antigen was found to be related to the bodies of
the organism.
Felix maintained that the granular agglutinins in the serum of a typhoid
patient bore a far closer relationship to the clinical course of the disease
than the ordinary floccular agglutinins, and could be used as an index of
the amount of immune bodies available for the patient's protection; and,
further, that an inoculated person was unprotected unless his blood
showed the presence of the heat-stable antigen agglutinins, or, in other
words, granular as well as floccular agglutination.
Felix himself failed to demonstrate any granular agglutination with the
sera of a number of inoculated men.
Arkwright came to the same conclusions working with the same group
of organisms, but along slightly different lines.
Working with a strain of *B. paratyphosus* A, which we may take as
representing the typhoid group, Arkwright distinguished four different
forms, namely: (a) a smooth-surfaced colony of motile bacilli; (b) a
smooth-surfaced colony of non-motile bacilli; (c) a rough-surfaced colony
of motile bacilli; (d) a rough-surfaced colony of non-motile bacilli.
The smooth colony of motile bacilli he regarded as the normal form of
the organism, and the smooth colony of non-motile bacilli as a very closely
related variant.
The rough colony of motile bacilli was a very well-marked variant, and
closely allied to it was the rough colony of non-motile bacilli.
For all practical purposes I think we may be allowed to regard the
organisms under discussion as having two forms, a smooth (normal) form
and a rough (variant) form.
He found that he was able to confer a far stronger immunity on guinea-
pigs against *B. paratyphosus* A by injection of a vaccine made of the
smooth form than when the rough variant was used.
By a series of agglutination tests between the smooth form and rough
variant of a certain strain of *B. paratyphosus* A and the immune sera
produced by them in rabbits, he was able to suggest a hypothetical antigenic
composition of these two variants.
He found:—
(1) A heat-labile antigen, common to both the smooth form and the
rough variant, which he called the "H" antigen.
(2) A heat-stable antigen peculiar to the smooth form which he called
the "S O" antigen.
(3) A heat-stable antigen, peculiar to the rough variant, which he
called the "R O" antigen.
On the strength of this he theorized that the greater protection con-
ferred by the smooth form must be connected with the presence of the
"S O" antigen, that is, the smooth heat-stable antigen, and not with the
"H" antigen, or heat-labile antigen, which is common to both.
Thus we see that Arkwright not only supports Felix's theory on this
matter, but carries it one step further by suggesting that the heat-stable antigen which causes the granular agglutination shall be of the smooth variety.

Arkwright also considers that a well-immunized person should show granular agglutinins in his serum.

Two quite definite provisos are thus suggested by these workers in connection with the production of a satisfactory vaccine:—

(1) That the vaccine must be made of the smooth form rather than of the rough variant of the required organism.

(2) That the blood of properly protected persons should agglutinate in a granular as well as in a floccular manner, thus demonstrating the presence of the theoretical heat-stable antigen agglutinin.

II.—When the first proviso was applied to the ingredients of the Army T.A.B. vaccine, it was not possible to say at once whether all the forms employed were smooth.

The forms of *B. paratyphosus* A and B were undoubtedly of the smooth variety, but the typhoid strain—Rawlins—had certainly a somewhat rough appearance.

Arkwright has four tests for determining whether a colony is rough or not, which are as follows:—

(1) The colony should have a rough, opaque surface with irregular edges.

(2) It should deposit in broth.

(3) It should auto-agglutinate.

(4) When emulsified and boiled it should give a granular agglutination with a serum produced by a rough variant, but no agglutination with a serum produced by a smooth form; and conversely, a boiled emulsion which gives granular agglutination with a serum produced by a smooth form must itself be smooth.

(The emulsion is boiled to remove the heat-labile antigen, which is supposed to be connected with the flagella of the bacilli; however, this appears to be done with greater precision by Bruce-White's method of treating the organisms with absolute alcohol for twenty-four hours at a temperature of 50° to 60° C. This latter method has been employed throughout the tests to be detailed further on, and the emulsions thus treated and deprived of their heat-labile antigens are called the "alcoholized emulsions" for the sake of brevity.)

To return again, then, to the Rawlins strain of *B. typhosus* which is used in making the Army T.A.B. vaccine.

Dr. Arkwright very kindly gave me a smooth form of a strain of *B. typhosus*, called "Mrs. S.," and when compared with this strain Rawlins certainly appeared decidedly rougher and deposited more quickly in broth. Rawlins did not, however, auto-agglutinate.

To apply Arkwright's last test, which he considers as the most reliable of them all, the following work was carried out:—

Three rabbits were given four intravenous doses of vaccine made with
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the Rawlins strain; three more rabbits were given similar doses of "Mrs. S." strain.

Ten days after their last injection their sera were examined against an emulsion of "Mrs. S.," killed with 0.2 per cent formalin, and also against an alcoholized emulsion of "Mrs. S."

The results of these agglutinations are given in Table I, from which it will be seen that those rabbits inoculated with "Rawlins" strain gave almost as good granular agglutination with the alcoholized "Mrs. S." emulsion as did the rabbits which had been inoculated with the "Mrs. S." strain, which is a known smooth form.

It may therefore be concluded that the form used for making the typhoid vaccine is a smooth one.

It will be seen that the sera of the Rawlins rabbits did not agglutinate to quite so high a titre as the sera of the "Mrs. S." rabbits, either in the floccular or the granular manner; it might seem therefore that "Mrs. S." is slightly the better antigen of the two.

The subsequent administration of a test dose of live B. typhosus to all six rabbits and two controls gave the following results:

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Weight (g)</th>
<th>Time of Death</th>
<th>Inoculated With</th>
</tr>
</thead>
<tbody>
<tr>
<td>757</td>
<td>2,350</td>
<td>24 hours</td>
<td>&quot;Rawlins&quot; vaccine</td>
</tr>
<tr>
<td>758</td>
<td>2,750</td>
<td>24 hours</td>
<td>&quot;Mrs. S.&quot; vaccine</td>
</tr>
<tr>
<td>762</td>
<td>3,000</td>
<td>24 hours</td>
<td>&quot;Mrs. S.&quot; vaccine</td>
</tr>
<tr>
<td>769</td>
<td>3,000</td>
<td>Alive</td>
<td>&quot;Mrs. S.&quot; vaccine</td>
</tr>
<tr>
<td>700</td>
<td>2,500</td>
<td>24 hours</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>761</td>
<td>2,075</td>
<td>24 hours</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>Control I</td>
<td>2,500</td>
<td>24 hours</td>
<td>Uninoculated</td>
</tr>
<tr>
<td>Control II</td>
<td>2,500</td>
<td>48 hours</td>
<td>Uninoculated</td>
</tr>
</tbody>
</table>

All the rabbits passed watery faeces after the test dose. B. typhosus was isolated from the gall-bladders of all those that died.

The small number of rabbits used, and the comparatively huge doses of typhoid organisms that have to be given as the test dose, precludes one from drawing any definite conclusions from these results, but it does appear that possibly the Rawlins strain, though of a smooth variety, may not be quite such a good antigen as, for instance, the "Mrs. S." strain.

III.—Turning now to the second proviso of a good vaccine, i.e., the production of granular agglutinins in the blood of the inoculated persons.

Three sets of sera were examined for the presence of the granular agglutinins; they were:

(a) The sera of rabbits inoculated intravenously with the vaccine (see Table I).

(b) The sera of men inoculated subcutaneously with the Army T.A.B. (see Table II).

(c) The sera of persons who were convalescent from an attack of typhoid fever (see Table III).

In the rabbits' sera there was a very considerable amount of granular agglutinins.

Only one amongst the inoculated men showed any granular agglutination.

And the typhoid cases not only showed no granular agglutination but the floccular agglutination was generally of a very low titre.
TABLE I.—THE AGGLUTINATION TESTS WITH THE SERA OF INOCULATED RABBITS.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Antigen</th>
<th>Agglutination titres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/150</td>
<td>1/250</td>
</tr>
</tbody>
</table>

A. Rabbits Inoculated with the "Rawlins" Vaccine.

Rabbit No. 757: B. typhosus "Mrs. S." + f + f + f + f
ditto alcoholized + g + g + g + g

Rabbit No. 758: B. typhosus "Mrs. S." + f + f + f + f
ditto alcoholized + g + g + g + g

Rabbit No. 759: B. typhosus "Mrs. S." + f + f + f + f
ditto alcoholized + g + g + g + g

B. Rabbits Inoculated with the "Mrs. S." Vaccine.

Rabbit No. 760: B. typhosus "Mrs. S." + f + f + f + f + f
ditto alcoholized + g + g + g + g

Rabbit No. 761: B. typhosus "Mrs. S." + f + f + f + f + f + f
ditto alcoholized + g + g + g + g

+ f = Floccular agglutination visible to the naked eye—Dreyer's technique.
+ g = Granular agglutination.
- = No agglutination.

TABLE II.—THE AGGLUTINATION TESTS WITH THE SERA OF MEN INOCULATED WITH T.A.B. VACCINE.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Antigen</th>
<th>Agglutination titres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/150</td>
<td>1/250</td>
</tr>
<tr>
<td>Pte. T., inoculated 8 months previously</td>
<td>B. typhosus &quot;Mrs. S.&quot;</td>
<td>+ f + f</td>
</tr>
<tr>
<td></td>
<td>ditto alcoholized</td>
<td>+ g + g</td>
</tr>
<tr>
<td>Pte. F., inoculated 10 days previously</td>
<td>B. typhosus &quot;Mrs. S.&quot;</td>
<td>+ f + f + f + f + f</td>
</tr>
<tr>
<td></td>
<td>ditto alcoholized</td>
<td>+ g + g + g + g + g</td>
</tr>
<tr>
<td>Major W., re-inoculated 10 days previously</td>
<td>B. typhosus &quot;Mrs. S.&quot;</td>
<td>+ f + f + f + f</td>
</tr>
<tr>
<td></td>
<td>ditto alcoholized</td>
<td>+ g + g + g + g + g</td>
</tr>
<tr>
<td>Pte. D., inoculated 10 days previously</td>
<td>B. typhosus &quot;Mrs. S.&quot;</td>
<td>+ f + f + f + f + f</td>
</tr>
<tr>
<td></td>
<td>ditto alcoholized</td>
<td>+ g + g + g + g + g</td>
</tr>
<tr>
<td>Corpl. S., inoculated 10 days previously</td>
<td>B. typhosus &quot;Mrs. S.&quot;</td>
<td>+ f + f + f + f + f</td>
</tr>
<tr>
<td></td>
<td>ditto alcoholized</td>
<td>+ g + g + g + g + g</td>
</tr>
<tr>
<td>Pte. N., inoculated 10 days previously</td>
<td>B. typhosus &quot;Mrs. S.&quot;</td>
<td>+ f + f + f + f + f + f</td>
</tr>
<tr>
<td></td>
<td>ditto alcoholized</td>
<td>+ g + g + g + g + g</td>
</tr>
<tr>
<td>Pte. C., inoculated 10 days previously</td>
<td>B. typhosus &quot;Mrs. S.&quot;</td>
<td>+ f + f + f + f + f + f + f</td>
</tr>
<tr>
<td></td>
<td>ditto alcoholized</td>
<td>+ g + g + g + g + g</td>
</tr>
</tbody>
</table>

+ f = Floccular agglutination visible to the naked eye—Dreyer's technique.
+ g = Granular agglutination.
- = No agglutination.
### TABLE III.—AGGLUTINATION TESTS WITH THE SERA OF PERSONS CONVALESCENT FROM AN ATTACK OF TYPHOID FEVER.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Antigen</th>
<th>Agglutination titres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/50</td>
</tr>
<tr>
<td>Banster, 8 weeks after onset</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>Patchin, 8 weeks after onset</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>Cairo case, ? date of onset</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>Cable, 12 weeks after onset</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>(Gravesend epidemic)</td>
<td>ditto alcoholized</td>
<td></td>
</tr>
<tr>
<td>Sanders, 9 weeks after onset</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>(Gravesend epidemic)</td>
<td>ditto alcoholized</td>
<td></td>
</tr>
<tr>
<td>Morris, 3 months after onset</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>(Gravesend epidemic)</td>
<td>ditto alcoholized</td>
<td></td>
</tr>
<tr>
<td>Victor Ward, 3 months after</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>onset (Gravesend epidemic)</td>
<td>ditto alcoholized</td>
<td></td>
</tr>
<tr>
<td>W. Ward, 3 months after onset</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>(Gravesend epidemic)</td>
<td>ditto alcoholized</td>
<td></td>
</tr>
<tr>
<td>M. Ward, 3 months after onset</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>(Gravesend epidemic)</td>
<td>ditto alcoholized</td>
<td></td>
</tr>
<tr>
<td>M. Smith, 3 months after onset</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>(Gravesend epidemic)</td>
<td>ditto alcoholized</td>
<td></td>
</tr>
<tr>
<td>I. Mason, 3 months after onset</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>(Gravesend epidemic)</td>
<td>ditto alcoholized</td>
<td></td>
</tr>
<tr>
<td>Holdstock, 3 months after</td>
<td><em>B. typhosus</em> &quot;Mrs. S.&quot;</td>
<td>+f</td>
</tr>
<tr>
<td>onset (Gravesend epidemic)</td>
<td>ditto alcoholized</td>
<td></td>
</tr>
</tbody>
</table>

Holdstock was inoculated several times during the war—the last time being in 1918. None of the other cases have ever received T.A.B. inoculations.

Holdstock’s serum agglutinated *B. paratyphosus* B up to 1 in 50, none of the other sera showed any agglutination with *B. paratyphosus* A or *B. paratyphosus* B.

*B. typhosus* was isolated from Cable’s faeces and Holdstock’s urine.
The same emulsion of formalinized "Mrs. S." and alcoholized "Mrs. S." was used for all three sets of agglutinations.

It would appear from the foregoing results that the persons convalescent from typhoid fever are in exactly the same state of immunity or non-immunity as the inoculated men, and neither of them are so well protected as the rabbits!

Felix demonstrated granular agglutination in the sera of typhoid cases by using a strain of B. typhosus which was particularly sensitive to the heat-stable antigen agglutinin. It seems, however, that Arkwright’s method of removing the part of the bacillus concerned with floccular agglutination and leaving the part associated with granular agglutination is far sounder than that of Felix, and, if his theories are correct, it should give an equally true result.

The small amount of antibodies, as represented by either floccular or granular agglutinins, in the blood of the typhoid convalescent cases, and the comparatively large amounts in the blood of the inoculated rabbits, are facts that can be much more easily explained by Besredka’s theories in connexion with what he calls "local immunity" than by the generally accepted theories that centre round the "antigen mosaic" idea.

Besredka’s theory is that immunity is conferred on the cells most affected by the disease, in this case the cells of the intestinal canal, and that the production of agglutinins in the blood is merely the reaction of the blood against a foreign body, and in no way a true measure of immunity to the disease.

He claims to be able to confer immunity without producing any agglutinins in the blood by giving the vaccine by the mouth.

In Conclusion.—After examining, in the light of Arkwright’s work, the cultures at present being used in making our T.A.B. vaccine, it is evident that they are all the smooth forms of the respective organisms.

This is all to the good, in view of the very strong evidence that Arkwright and others have brought forward of the superiority of the smooth over the rough forms in their power of conferring protection.

Whether, however, we are justified in taking Arkwright’s theories as a working hypothesis in our attempts to estimate the immunity conferred by a vaccine is somewhat open to doubt.

My thanks are due to Lieutenant-Colonel Gray, R.A.M.C., for allowing me to publish these notes, to Dr. Arkwright for his assistance and advice, and to Dr. Outred and Dr. Ponder for permitting me to investigate their cases and for putting their clinical and laboratory findings at my disposal.

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