Preliminary Note on Immunization Against B. Paratyphosus A.

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In the Journal of the Royal Army Medical Corps for August, 1912, Colonel R. H. Firth, writing on the subject of the prevalence of enteric and paratyphoid fevers in India, makes the following statement:

"The disturbing factor is the prevalence of paratyphoid fever, against which disease anti-enteric inoculation appears to have little influence. This view is not new, but emphasizes the plea for a bi-valent emulsion with which inoculation must be carried out against both diseases."

Some time ago, at the request of Lieutenant-Colonel Sir William Leishman, we initiated experiments with a bi-valent emulsion of B. typhosus and B. paratyphosus A, using groups of rabbits on the same lines as those employed by Sir William Leishman and his co-workers in their work on antityphoid vaccine.

These experiments are still going on, and it is not proposed, in this paper, to anticipate in any way the final conclusions of the research. The work which we now publish is only brought forward...
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because it appears to us to throw an interesting side-light on some of the clinical findings in paratyphoid A fever. It may also be taken to show that the question of prophylaxis against this disease is more complex than might at first sight appear to be the case, and may perhaps emphasize the necessity of a thorough experimental basis before an anti-paratyphoid A vaccine is finally recommended for the use of troops.

In our first experiment with a bi-valent emulsion we were surprised to find that, although satisfactory production of immune substances for B. typhosus followed the injections, the serum of our rabbit-groups showed only very slight response for B. paratyphosus A. In the case of the latter organism we failed to obtain agglutination in higher dilution than 1 in 60, and our experiments failed to elicit evidence of opsonins or bactericidins. Although Besson has mentioned the impossibility of producing immunity to B. paratyphosus A in rabbits, we had evidence that his statement requires revision, as we had, in the laboratory, a rabbit serum capable of agglutinating this organism in a dilution of 1 in 2,000. This serum, kindly given us by Dr. Leadingham, of the Lister Institute, afforded a proof that rabbits can be immunized against B. paratyphosus A. It seemed probable that our experiment had failed through the use of insufficient dosage. Our rabbits had received a first dose of 1 c.c. and a second dose of 2 c.c. of a mixed vaccine containing 25 millions of each organism per c.c., or, in other words, a total of 75 millions of each germ, the weight-for-weight equivalent of the 1,500 million typhoid bacilli used in anti-enteric inoculation in man. Dr. Leadingham, to whom we applied for information, was good enough to look up the records of his serum, and told us that the rabbit producing it had received a series of six injections, first with killed, then with living cultures of B. paratyphosus A, amounting to a total of 3 c.c. of a saline emulsion of an agar slope. Various "counts" have led us to regard an emulsion of one agar slope of B. typhosus in 10 c.c. of saline as containing roughly 2,000 million bacilli per cubic centimetre and the same estimate will not be far out for B. paratyphosus A, which grows on agar with much the same facility as B. typhosus. It is probable that Dr. Leadingham's rabbit received something like 6,000 million bacilli in all, as compared to our 75 million, which would adequately explain our failure. But doses of equivalent amount for man would probably

cause a very severe reaction and are hardly likely to be used in practice. The point of real importance was that, given in equal doses, B. paratyphosus A appeared to be a much less efficient antigen than B. typhosus.

In September, 1911, we started a fresh series of experiments designed to ascertain what dose of B. paratyphosus A would produce agglutinins to a degree comparable with the agglutinin production following the usual prophylactic dose of B. typhosus,

or, rather, its equivalent for rabbits. For this purpose we used three, and finally four, groups of rabbits, three animals to each group, the weights of the different groups being equalized as far as possible. Group I was kept as a "control" and received no vaccine. Group II received a series of increasing doses of a bi-valent emulsion of B. typhosus and B. paratyphosus A. Group III was given similar doses of B. typhosus alone, while Group IV was treated with two inoculations amounting to 1,500
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millions of *B. paratyphosus* A. All the vaccines had been killed by heat (53 °C), and subsequent addition of lysoL It may be mentioned here that the first experiment had contained a fallacy, inasmuch as the paratyphoid A vaccine used had been heated to 60° C. for half an hour, while the typhoid vaccine had been killed as usual at 53° C., so that the two were not strictly comparable. Chart I, however, shows that the agglutinins for paratyphoid A were greatly below those for typhoid in the second experiment as in the first.

A point of great interest emerges in Chart II, where the typhoid agglutinins produced in Group II and Group III are compared. It is here seen that the typhoid agglutinins were consistently higher in the group of rabbits immunized with a mixed vaccine than in the group immunized with *B. typhosus* alone, although the doses of this organism were the same in both. Further, in Chart III, where the paratyphoid A agglutinins in Group II and
Group IV are compared, it is seen that these were decidedly less after use of the mixed emulsion than after the two doses of paratyphoid A vaccine alone. This latter fact may perhaps be due to a diminished response on the part of the tissues after a long series of inoculations, as it is seen in Group III that the two last inoculations of 1,000 millions each led to but a very small response, in fact, failed to check the gradual drop of immunity. But, on the other hand, the better production of agglutinins by an unmixed paratyphoid A vaccine as compared to that by a bi-valent emulsion may be a phenomenon complementary to that exhibited in Chart III for B. typhosus.

A third observation was instituted in April, 1912. On this occasion separate groups of rabbits were employed for each organism and the results compared. As before, Group Ia was retained as a control and given no treatment. Group IIa received 25 millions B. typhosus on April 11 and 50 millions on April 22.
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Group IIIa received similar doses of B. paratyphosus A on the same dates. The serum was invariably collected twenty hours after the blood had been withdrawn and was heated to 58° C. for twenty minutes before testing. This heating was carried out to eliminate "complement," as this substance is difficult to estimate by quantitative tests, probably varies in the same serum at different times, and thus introduces a fallacy into examinations of sera for immune substances. Known quantities of guinea-pig serum were employed to "complement" the heated rabbit sera in testing for bactericidins.

The agglutinin production is shown in Chart IV, and again the agglutinins for B. typhosus are much more marked than for B. paratyphosus A. The thermo-stable opsonins, calculated by Klien's ¹ method of dilution to an end-point, are shown graphically in Chart V, where the number of bacilli per phagocyte is shown for each dilution by the horizontal measurement of the shaded area at each level, one degree of the scale corresponding to one bacillus per phagocyte.

The chart shows how much greater the phagocytosis becomes after immunization with B. typhosus than after immunization with B. paratyphosus. (A) Thermostable Opsonins for B. typhosus (A). Thermostable Opsonins for B. paratyphosus (A).
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The opsonins for B. typhosus are rendered in half-tone, those for B. paratyphosus A in black. It is seen at once that while the typhoid opsonins in Group II. are well marked, the opsonins for paratyphoid A in Group III. are hardly significant.

It should be added, in explanation of the chart, that the "end-point" is taken as one bacillus per phagocyte, not 0·5 bacilli as advised by Klein. This was done as, on one occasion, we counted as many as 0·98 bacilli per phagocyte in the heated serum of the control group. Again, finding it difficult to count accurately more than twenty-five bacilli in a phagocyte, we decided to take this as our maximum, and all "uncountable" cells were taken as containing twenty-five bacilli. On April 29 the 1 in 3 dilution of serum brought about a phagocytosis that was actually greater than is shown on the chart in the case of both organisms, and on May 14 this was also the case for B. typhosus even in a dilution of 1 in 15. The chart brings to light the curious fact that the end-point may be very high and yet the degree of phagocytosis in low dilutions be comparatively small. This apparent anomaly has been since noticed in other observations of the same kind, and is not merely accidental. It may possibly be explicable on the hypothesis that there are two thermo-stable bodies concerned in phagocytosis—an idea which receives support from the highly interesting work of H. R. Dean on the complex nature of agglutination. Attempts to calculate the bactericidal action gave rise to irregular results, and we failed to obtain deviation of "complement" with bacterial emulsions "sensitized" with the heated sera.

It is of interest to compare our results with the findings of Majors Grattan and Harvey, Colonel Firth and others in actual cases of paratyphoid A fever. The very low degree of agglutinin production for the homologous organism and the frequent presence of non-specific agglutinins for B. typhosus in the blood of cases of paratyphoid A fever is comparable to our failure to produce high agglutination-titres in experimental animals for B. paratyphosus A, and the curious over-production of typhoid agglutinins by a bi-valent vaccine as compared with the agglutinins in animals inoculated with typhoid vaccine alone.

It would be interesting to know whether all cases showed both paratyphoid A and typhoid agglutinins, or whether the latter only arose in patients previously inoculated against typhoid. In rabbits

immunized with paratyphoid A vaccine only we were unable to demonstrate any agglutination of typhoid bacilli. Another matter of great interest is the fact, mentioned by Colonel Firth, that no less than 14 per cent of cases of paratyphoid A fever become “carriers” for more or less protracted periods, as opposed to 2 per cent in the case of typhoid. It is tempting to connect this with the low titre of bacteriotropic substances recorded both in cases and in experimental animals, a condition which would appear to favour the permanence of bacterial foci in the tissues after the septicæmia has been overcome.