Gastrostomy was performed in the first case on the suggestion of Colonel D. O'Sullivan, C.M.G., A.M.S., and to his suggestion both patients owe their lives. Cervical oesophagotomy should be a perfectly safe operation if gastrostomy is subsequently performed, but without it a recovery could hardly be looked for, owing to the extremely foul character of the discharge from the oesophageal opening, which would naturally be greatly accentuated by the leakage of food from the oesophagus.

I have to thank Dr. Coady, of Kildare, for his kind assistance in the first case, and Captain W. C. MacFetridge, R.A.M.C., in the second.

SUMMARIZED RESULTS AND OBSERVATION FROM AGGLUTINATION AND ABSORPTION TESTS BY THE TIME-GOVERNED SLIDE METHOD.

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The following summarized results and observations made during the war, from the practical application of my time-governed slide method for the agglutination test in cases arising in France, Gallipoli, Salonika, and Malta form an appendix to the publications of the technique of that method in the Journal of the Royal Army Medical Corps, and may present points of particular or general interest to fellow workers. The test was carried out whenever possible and as part of a system of complete examinations on cases receiving laboratory investigation.

Bacillus Typhosus Vaccine.

During the years 1914-15 almost all patients or subjects examined had been inoculated with antityphoid vaccine. Of the sera tested all but one inoculated within two years showed specific agglutinin action on the B. typhosus.

B. paratyphosus A and B Vaccine.—Nearly 3,000 subjects were inoculated in Malta during 1915 and early in 1916 with B. paratyphosus A and B, treated with normal serum, vaccine made as I have described (British Medical Journal, August 8, 1914). The sera tested from a certain number thereof gave positive agglutination of both micro-organisms up to six months, and with B. paratyphosus B up to ten or twelve months after inoculation. Following anti-paratyphoid vaccine the agglutinin action for B. typhosus due to previous inoculations with the antityphoid vaccine was frequently increased.

T. A. B. Vaccine.—In subjects inoculated with T. A. B. vaccine it has been noticed that, as a general rule, agglutinin for B. paratyphosus A had diminished or disappeared first in point of time, whilst that of B. paratyphosus B remained longer, although the converse has been noted. That for B. typhosus remained the longest time.

1 The previous papers appeared in the November and December, 1919, issues.
Enterica Infections.

Appearance and Disappearance of Agglutinins.—Specific agglutinin action arising from infection was present in the sera of patients towards the end of the first week of illness, except in occasional cases due to B. paratyphosus A, wherein it was not determined till the second week, while in one case it was not determined till the twenty-third and in another till the thirty-fourth day after the onset. Specific agglutinin for B. paratyphosus A developing in disease, when compared with the specific agglutinin developing for B. paratyphosus B, and especially when compared with that in B. typhosus infections, tends to disappear earlier from the sera, especially in short febrile cases; the variation in degree of paratyphosus A agglutinin action is great; occasionally it has been not only transitory but very small. The low dilutions used in the slide method have been helpful in detecting it.

Non-inoculated.—Enterica patients, who had not been inoculated frequently, gave sera that agglutinated within four minutes that emulsion only which corresponded to the micro-organisms with which they were infected.

When co-agglutinin action appeared, it was always later on in infections and these were almost all cases due to B. typhosus. Co-agglutinins did not produce agglutination as quickly in point of time, and their action was but rarely determined within four minutes in as high dilution of sera as the specific agglutinins. Only exceptional sera of cases due to B. paratyphosus B or B. paratyphosus A developed detectable co-agglutinins.

Cases inoculated with Anti-typhoid Vaccine.—Patients who had received anti-typhoid vaccine and were infected with any micro-organism of the enterica groups gave sera wherein the specific agglutinin for B. typhosus already present was, as far as one can judge, increased. When the cases could be examined early in their infections the results were simple of interpretation. Those due to B. typhosus gave sera that agglutinated only the emulsion of B. typhosus, while those due to either B. paratyphosus A or B gave sera that agglutinated the emulsion corresponding to the infecting micro-organism as well as the emulsion of B. typhosus. When tested late in the second or third week of the disease, cases due to B. typhosus not infrequently gave sera that temporarily showed slight co-agglutinin action for B. paratyphosus A and B. But such action was not as rapid nor detected in such high dilutions of sera tested on the slide as that of the specific agglutinin for B. typhosus. When tested in the second or third week of the disease cases due to infection with B. paratyphosus A or B appeared to increase the inoculation agglutinin content of sera for B. typhosus, especially when the infection was due to B. paratyphosus B. One group of the B. paratyphosus very rarely produced any co-agglutinin action in the sera for the other paratyphosus group.

The army routine measure of interval agglutination examinations which I have carried out previous to and from the opening of the laboratory of the first infectious diseases hospital in France (14th Stationary) in 1914 have shown almost constantly the specific agglutinin curves to be more prolonged.

Inoculated T. A. B. Vaccine.—In considering cases of infection with one of the three groups, one has had to make note that the sera of non-infected subjects do not give a corresponding reaction at any defined time after the T. A. inoculation. Repeated interval examinations are essential to diagnosis, and one
Clinical and other Notes

has had to determine the time or titre curves marking the rise or fall of an agglutinin action in order to determine serologically the infection present.

Irregular Findings.—These are infrequent; but their incidence may be in part comprehended for reasons given in Article I and by the fact of the known variation in minor characters to be found amongst strains of a group of micro-organisms and the possibility of concurrent infections. In the typhoid group two uninoculated patients gave in the third week of their disease and just prior to death sera that failed to agglutinate the B. typhosus emulsion by the long method. The serum of another similarly uninoculated case dying in the third week of typhoid fever agglutinated, equally on the slide and by the long method, both emulsions of B. typhosus and B. paratyphosus B. Careful search failed to find B. paratyphosus B in association with the B. typhosus which was isolated before and after death. A fourth case that died and was uninoculated gave serum that did not agglutinate, in any of the several dilutions tried, the B. typhosus isolated, but did agglutinate the stock emulsion and several other strains of B. typhosus. The strain isolated was readily agglutinated by sera from five other patients suffering from typhoid fever. In the paratyphoid B group, two cases gave sera that transitorily agglutinated the emulsions of B. paratyphosus A and B. paratyphosus B in equal high dilution by a long method; though in point of time agglutination of the latter emulsion took place just before that of the former in the dilutions tested on the slide. One anti-typhoid inoculated case, from which B. paratyphosus B could alone be recovered from the blood, gave serum that, examined three times at seven days interval, did not agglutinate by my slide or by the long method in any of the several dilutions employed the emulsion of B. paratyphosus B, but agglutinated readily in low and high dilutions those of B. typhosus and B. paratyphosus A. The strain isolated had the fermentation, agglutination, and absorption character of a typical B. paratyphosus B. Two cases, of interest rather for a possible than a real danger in interpretation, gave sera that early after a clinically diagnosed relapse of B. paratyphosus A infection, when a B. paratyphosus B was isolated from the blood, agglutinated only the stock emulsions of B. typhosus (due to inoculation) and B. paratyphosus A and not that of B. paratyphosus B nor the strain isolated.

A week later in each case the specific agglutination of the B. paratyphosus B of the stock emulsion and of the strain isolated was detected. Absorption test on the above serum showed the agglutination to be due to specific agglutinins. In the paratyphoid A group, several cases gave sera that showed throughout the disease and convalescence agglutinin action for B. typhosus, because of previous anti-typhoid inoculation, more rapidly and to higher dilutions than that for B. paratyphosus A, although the latter bacillus was recovered from the bloods. In two other cases no specific agglutinin action for the strains of B. paratyphosus A, recovered from the blood, stock emulsion or two other strains of B. paratyphosus A, could be determined, during the illness or convalescence, by either my slide or the longer method.

B. Dyssenteric Infections.

When the disease was due to B. dysenteriae Shiga or Flexner-Hiss, the associated agglutinins in the sera, due to previous anti-enterica vaccination, were determined throughout the disease and, as far as one could judge,
were unaffected by the \textit{B. dysenteriae} infection. The specific agglutinin content present, or its later development in sera of bacillary dysentery patients, was not apparently influenced by antibodies formed in response to the injections of anti-dysenteric sera.

Double infections with \textit{B. dysenteriae} Shiga and \textit{B. dysenteriae} Flexner-Hiss have been indicated by the agglutination test and have been confirmed by the absorption test and in two cases proven bacteriologically.

\textit{B. dysenteriae} Shiga.—Specific agglutinin action for \textit{B. dysenteriae} Shiga in sera generally appeared between the seventh and sixteenth day following the onset of acute symptoms, rarely earlier. In two per cent of all cases no agglutinins were detected. A lesser co-agglutinin action on \textit{B. dysenteriae} Flexner-Hiss was detectable in about one-fourth of the cases.

The presence of detectable specific agglutinins in sera appeared in general to be dependent on the length of time the bacilli were acting pathogenetically. This agglutinin action not infrequently early disappeared. It occasionally persisted for some months and in many of these cases the acute symptoms in the intestine or nerve or joint complications were prolonged.

In a limited number of cases followed from the first or second day after onset, it appeared that when frequent salines were given alone or with anti-dysenteric serum early after the onset, and the temperature was raised only for three or four days, specific agglutinins were generally only slight and occasionally only transitory in the second or third week, though the onset was most acute and \textit{B. dysenteriae} Shiga very numerous in the stools during the first few days.

The sera of two cases, examined post-mortem and found to have no marked macroscopic lesions in the intestine, gave a positive agglutination. The sera of four cases, who died early in the disease and had extensive typical macroscopical lesions, gave no agglutination. \textit{B. dysenteriae} Shiga was isolated from all six.

\textit{B. dysenteriae} Flexner-Hiss.—A selected stock strain of \textit{B. dysenteriae} Flexner and one of \textit{B. dysenteriae} Hiss have been frequently employed simultaneously in the test mainly for interest and instruction. The difference of degree in which they are agglutinated in positive cases due either to \textit{B. dysenteriae} Flexner or Hiss is slight. For diagnostic purposes the strain of \textit{B. dysenteriae} Hiss "J" now in use for nearly two years is very reliable, and has proved itself the only reliable one from amongst many strains of the \textit{B. Flexner-Hiss} group that were comparatively examined.

The variation in severity and frequent mildness of dysenteric symptoms in cases due to \textit{B. dysenteriae} Flexner-Hiss makes it difficult to determine what date after onset the agglutinin action appears.

Serum taken prior to and at the autopsy of two cases due to strains of the \textit{B. dysenteriae} Flexner-Hiss group did not agglutinate the stock emulsions nor the strains isolated. The lesions seen in the intestines were those seen in the severe type of bacillary dysentery. Death occurred early in the disease in each case.

Very rarely there is isolated from the feces of a dysenteric patient a bacillus which corresponds in biological characters to typical strains of the Flexner-Hiss group and produces an agglutinin specific for itself.
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M. MELITENSI S AND HETERO-AGGLUTININ ACTION IN SERA.

Sera of patients suffering with undulant fever agglutinated also the microorganisms corresponding to those previously given in prophylactic vaccines, but no rise in the titre of such sera for them was apparent.

On account of the variable prodromal symptoms in melitensis infections, the date of onset of the disease is difficult to determine. In over thirty cases the specific agglutinin action for *M. melitensis* was found present in the sera at the first tests made soon after admission to hospital. In two mild cases admitted very late in convalescence the agglutinin action for *M. melitensis* had disappeared, while that produced by anti-typhoid vaccine inoculation persisted.

Hetero-agglutinin for *V. cholerae* was present in the sera prior to true convalescence in the great majority of cases systematically examined from early after the onset of the fever. This hetero-agglutinin action was occasionally determined in the urines when also present in the sera, and only when the specific agglutinin action for *M. melitensis* was marked in the sera and also present in the urines. Absorption tests confirmed the findings.

During the fever a very transitory and slight agglutinin action for *B. dysenteriae* Flexner-Hiss in two sera and for *B. dysenteriae* Flexner-Hiss and *B. dysenteriae* Shiga in one serum diluted 1 in 10 was noted. Hetero-agglutinin action was again confirmed by the absorption test.

The co-agglutinin for *M. paramelitensis* was found present to a very slight degree in rare cases, and only when the specific agglutinin was marked in its action.

In all cases and at all stages of the disease, the specific agglutinin was the first to show its action by the slide method.

As some authors have found a difference of action between non-heated sera and sera heated sufficiently to destroy its complement, a series of tests were made on several patients' sera. Sera tested before and after heating showed no difference in their agglutinin action on the strain used. Though the strain of *M. melitensis* has been systematically used when testing the unheated sera of patients suffering with any of many febrile diseases, it has never been agglutinated in 1 in 10 dilution of unheated serum within three minutes by my slide method unless the patient has undulant fever.

After following several cases for some six months or more, I have observed that late in true convalescence the hetero-agglutinin action for *V. cholerae* was not detected and the specific agglutinin for *M. melitensis* fell rapidly in titre, but in two cases only had it disappeared after two to three months.

It must be remembered that the emulsion of *M. melitensis* to be used in the slide test must be very dense in order to avoid an inhibition zone, which is occasionally seen even in a 1 in 10 and 1 in 20 dilution, should a thinner emulsion (of a density such as that of the other emulsions) be employed.

**V. CHOLERÆ.**

During the War I have found only one serum from an uninoculated man that has shown specific agglutinin action for *V. cholerae*. He had come late from Serbia and was a foreigner. His serum did not agglutinate *M. melitensis*. In the
absence of experience of the time-governed test on many cholera infected patients I am not able to say if a 1 in 6 dilution of serum can be employed with more advantage than the 1 in 10 dilution.

Zones of Inhibition.

During the comparative testing of many sera, a few gave a specific and clearly positive result in a 1 in 10 dilution when the standardized emulsion was used and not when a thin emulsion was used. These examinations were made with sera from one man recently inoculated against typhoid, from two cases of typhoid fever, from one case of paratyphoid A fever, and from three cases of undulant fever. The sera from these cases showed similar negative results after twenty-four hours' contact with their corresponding emulsion in a 1 in 10, 1 in 20 and 1 in 40 dilution, but agglutinated it in a 1 in 80 and 1 in 160 dilution.

The zone seen present when using a thin emulsion of M. melitensis was not constant in the serum, for in two cases in the second examination of the sera, made within a few days, this zone was no longer present. There was no apparent dependence of the zone upon the duration or the severity of the infection.

Varied Agglutinability of Separate Colonies and of Strains.

The experiences such as the following are rare, but have an interest when strains are being selected for working upon, and when subcultures are being made.

A single colony of B. dysenteriae Shiga that grew from a stool plating on Conradi's medium was replated on to the quarter area of another plate of the same medium to secure pure isolated colonies. One of the isolated colonies on plate 2 was agglutinated before and after successive sub-cultures on agar in a 1 in 50 dilution of anti-typhoid, anti-paratyphoid A and B, anti-dysenteric Shiga and Flexner sera (Lister Institute), and 1 in 10 normal human serum within two minutes. Another colony tested in similar dilutions of the same sera was only agglutinated by the anti-dysenteric serum, Shiga. Both colonies showed the morphological, fermentative and absorption characters typical of B. dysenteriae Shiga. Again on examining isolated colonies from plating out a strain frequently subcultured and tested from time to time during some years, one colony was agglutinated in anti-Shiga and anti-Flexner-Hiss animal sera and not in other anti-sera tested nor in normal serum, while another colony was agglutinated in anti-Shiga serum only. These properties persisted in several subcultures made and tested.

The paratyphosus A. strain used in Professor Widal's laboratory and kindly given to me six years ago is employed in the test, as no better strain has been found for agglutination work. Once, some three years ago, it became inagglutinable, and the bacilli had grown up as cocci-bacilli, but the colonies isolated from an inoculation abscess following its injection were upon culture readily agglutinated. Recently on slightly dry media it grew in coccal form and was almost inagglutinable. Its bacillary form and excellent agglutination properties returned after twice subculturing the growth in agar tube condensation water or broth and then on moist agar tubes.

Most grateful thanks are extended to my collaborators and assistants who have worked with me during the long series of investigations which are herein summarized.