LABORATORY RESEARCH ON BACILLARY DYSENTERY.

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In a recent contribution by Dr. Manson-Bahr to the "Correspondence Circle" of this Journal on the subject of research on the more common tropical diseases the following important points in the laboratory examination of cases of bacillary dysentery are mentioned as worthy of further observations: (a) The persistence of dysentery bacilli in the faeces of convalescents; (b) the value of serum reactions in the diagnosis of the acute and chronic disease.

There is little doubt that more extensive study of these questions, and indeed of every aspect of this disease, cannot but lead to its better understanding. At the same time the impression may be formed by perusal of the note referred to that the knowledge we already possess on the above points is meagre. A brief account, therefore, of the writer's experience of these problems, much of which has been published, may be of interest and assistance to those contemplating the pursuit of these investigations.

THE PERSISTENCE OF THE DYSENTERY BACILLI IN THE Faeces DURING THE CONVALESCENT STAGE OF THE DISEASE AND DURING THE "CARRIER STATE."

Much information on this question is embodied in an article on Bacillary Dysentery by Manson-Bahr and the writer in "The Practice of Medicine in the Tropics" (Byam and Archibald).

The incidence of the carrier condition has been investigated by various observers in several large groups of cases, and has been found to vary from three to seven per cent. The duration of the period of infectivity of convalescents has, in the writer's experience, been very largely dependent on the clinical condition of the patient. It is possible, of course, that the mildest case may be a potential carrier, but it was found that the period of elimination of dysentery bacilli was most often prolonged in the chronic relapsing type of the infection. In these latter cases clinical evidence of some chronic ulceration of the bowel was obvious, and amelioration of the symptoms coincided with the disappearance of the organisms from the stools.

Observations on this point, extending over a period of twelve months were made by the writer at the Central Dysentery Hospital established in England for the reception of these cases from abroad. From the data obtained by these investigations the following table (from "The Practice of Medicine in the Tropics") was compiled, and is illustrative of a large series of cases in the "carrier state."

<table>
<thead>
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<th>CHRONIC CARRIERS OF Bacillus dysenteriae SHIGA. 67 CASES.</th>
<th>Per cent.</th>
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<tr>
<td>Positive four months from onset</td>
<td>77</td>
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<tr>
<td>,, six months from onset</td>
<td>19</td>
</tr>
<tr>
<td>,, twelve months from onset</td>
<td>4</td>
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In the very great majority of the cases included in the above table the persistence in the stools of small quantities of mucus was evidence that the chronic inflammatory condition of the bowel had not entirely subsided. Beyond an occasional mucoid stool, however, the patients were in fair health and actual typical relapse was infrequent. Briefly, the clinical condition was that which has been termed by Cunningham "latent dysentery." In such cases bacteriological examination of the stools yielded most irregular results, positive and negative examinations alternating, or occasionally a positive result following a series of negative indicated the continued infectivity of the patient. This intermittent elimination of the infecting organism discounts to an important degree the value of a series of negative bacteriological examinations as indicating the non-infectivity of any dysentery convalescent.

A macroscopic and microscopic examination of the stool is easily and rapidly made and will indicate the presence or absence of mucus, or of mucus and blood. The result of such an examination may be accepted as a useful criterion as to whether the convalescent is a potential source of danger to the community. This method is undoubtedly the only practical way of dealing with the question when the investigation of large numbers of individuals has to be undertaken.

From the foregoing remarks it will be evident that the experience gained in the examination of cases in this stage of the disease suggests that although the "carrier" of dysentery bacilli, especially of *Bacillus dysenteriae* Flexner, may present symptoms so mild as to be readily overlooked, his condition of health could not be described as normal.

**Serum Reactions in the Diagnosis of Bacillary Dysentery.**

The sero-diagnosis of bacillary dysentery is also suggested as a subject for re-investigation by the macroscopic technique of agglutination. The fact that the macroscopic method is emphasized in the note to which reference has been made may possibly create the impression that all previous investigation of serum reactions in these infections has been undertaken by the now archaic microscopic method of applying this test. It is hardly necessary to remind laboratory workers who have passed through the classes at the Royal Army Medical College that the technique which should be adopted is the macroscopic, a method with which they are thoroughly familiar. It may be helpful, however, to those investigating this aspect of the disease to record shortly the writer's experience of this method of examination in infections with *B. dysenteriae* Shiga and the various serological strains of *B. dysenteriae* Flexner.

The observations about to be recorded were made in the closing phase
of the war at a period when bacillary dysentery, mainly of the Flexner type, constituted a serious cause of disability and invalidism. Opportunity of continuing these observations in the more chronic type of the infection was afforded by the examination of a large number of convalescents at the Central Dysentery Hospital subsequent to the armistice.

**The Period of the Disease at Which Agglutinins Appear in the Serum.**

Cases came under observation at stages of the disease varying from the fourth to the thirty-second day from the onset. The presence of demonstrable agglutinins was noted in some cases as early as the fifth or sixth day, but more commonly after the tenth day.

In all cases the tests were made with standard agglutinable emulsions of the various dysentery bacilli and, in addition, in many instances with formalinized broth cultures of the actual organism isolated from the patient.

**The Quantitative Estimation of Agglutinin Production.**

In making any accurate observations relative to this point the employment of standardized agglutinable emulsions is obviously a very great advantage. In the absence of standardized suspensions, formalinized broth cultures made from type strains of the various organisms and diluted down to the required opacity, will be found to yield most useful and consistent results.

It may be stated at once that the titre of the patient's serum in which positive agglutination of the infecting organism is evident is always low and is in no way comparable to that seen in typhoid or paratyphoid infections. The degree of agglutinin production in bacillary dysentery is largely dependent on the clinical type of the case, and on the identity of the infecting dysentery bacillus. In general, the more severe the symptoms developed during the course of the disease the higher was the serum dilution in which a positive reaction was evident, and Shiga infections invariably yielded more powerful reactions than infections with the mannite-fermenting group of bacilli.

Passing from these generalizations, the following brief details are illustrative of the results obtained in the serological investigation of these cases. Those more especially interested in this question should refer to the Medical Research Council Special Report Series, No. 42, p. 45, where specific details of the serum reactions of a group of cases infected with the mannite-fermenting bacilli are recorded.

*In Shiga infections* the titre of the serum in which positive agglutination occurred was frequently as high as 1 in 250, exceptionally 1 in 500, and in rare cases 1 in 1,000. No case of Shiga dysentery, proved by actual isolation of the organism, failed to give positive evidence by the production of agglutinins of the existence of the infection. In the milder type of case clumping of the suspension might be evident only in dilutions
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as low as 1 in 25, but as the test always included a range of dilutions of from 1 in 25 to 1 in 250, positive results were not missed.

It has been suggested that "normal" sera, i.e., sera of individuals who have not suffered from a Shiga infection, may agglutinate suspensions of this bacillus in low dilution. This fallacious result has not been experienced, and provided that the agglutinable emulsion employed in the test is selected and controlled in the usual manner, reliance can be placed on positive serum reactions in Shiga infections.

There are, however, a few precautions which must be observed in interpreting the result of a positive reaction. The history of the patient must be carefully ascertained, and the occurrence of a previous Shiga infection must, if possible, be excluded, as agglutinins for the organism may persist in the serum for some years subsequent to the infection. In several individuals, from whom the history of a previous attack of Shiga dysentery was elicited, the writer has found the serum to give a positive agglutination reaction in a dilution up to 1 in 125 for as long as from two to three years following the original infection. The fact that an anti-dysentery serum is commonly administered to these cases at an early stage in treatment should also be borne in mind and the patients' serum must be obtained before the injection of Shiga anti-toxin. The reasons for this precaution are, of course, obvious.

In Flexner dysentery the application of the agglutination test is complicated by the fact that several closely related serological strains of mannite-fermenting bacilli may be responsible for the infection. Agglutinable emulsions for these various organisms can, however, be readily prepared and the serum tested against each strain.

A negative reaction cannot be assessed at as high a value as has been noted in infections with the Shiga bacillus. Whereas in the latter type of dysentery a serum test has invariably yielded positive evidence of agglutination, in the case of the mannite-fermenting bacilli twenty to thirty per cent of the cases examined failed to demonstrate the production of agglutinins for the infecting bacillus. It has been noted that the extent to which agglutinins are elaborated is dependent on the severity of the infection. The explanation, therefore, of these negative reactions apparently lies in the fact that in certain cases the symptoms produced by the Flexner group of bacilli may be mild and transient in nature; it is a commonplace that they are frequently diagnosed clinically as "diarrhoea."

In positive reactions the titre of the serum in which agglutination of the infecting organism is evident, whilst varying with the severity of the infection, is, in general, low. In a series of cases proved by isolation of the V-strain of bacillus, positive reactions were noted in dilutions of from 1 in 25 to 1 in 125. Although agglutination might occasionally occur in a dilution of 1 in 250 or over, cases yielding these more marked reactions were uncommon.

The agglutination of one or other of the mannite-fermenting bacilli by "normal" sera, i.e., sera of individuals who do not give a previous history
of Flexner dysentery, must be noted as complicating the interpretation of the test in these infections. This phenomenon is not infrequently observed if the agglutination reactions of the sera of a number of healthy individuals are investigated. The probable explanation of these results is that a previous Flexner infection has occurred, but has been so mild that a bacteriological examination of the faeces has not been made and the true nature of the case has passed unrecognized. A possible alternative explanation is that such individuals may have ingested subinfectious doses of these organisms and, although actual infection has not resulted, demonstrable antibodies have been produced. Evidence in support of both these hypotheses has been placed on record by several observers, and the writer's experience is that agglutination of the mannite-fermenting strains by so-called "normal" sera is to be observed most commonly in endemic areas of these infections.

A further point relative to the presence in the serum of "normal" agglutinins for the Flexner bacilli is worthy of notice. It can frequently be determined that in pure Shiga infections, that is to say cases in which careful and repeated bacteriological examination of the stools has resulted in the isolation of *B. dysenteriae* Shiga only, in addition to the production of agglutinins for this organism, any Flexner agglutinins present are markedly increased. The phenomenon is somewhat analogous to the commonly observed fact that paratyphoid infections in patients who have previously been inoculated with a *typhoid* vaccine usually causes a rise in titre of the typhoid inoculation agglutinins. Unless the possibility of this occurrence is recognized an incorrect diagnosis of a mixed infection might be made on the result of a serological test which indicates the presence of both Shiga and Flexner agglutinins.

In cases of chronic dysentery a serological examination should always be made, as valuable information suggestive of a previous bacillary infection may be obtained. More especially is this the case in Shiga infections, for residual agglutinins for this organism may, as has been previously noted, persist in the serum for years. It has recently been the practice at the Queen Alexandra Military Hospital, London, to test the serum of chronic and relapsing cases of dysentery in which, as is usual, unsuccessful attempts have been made to isolate a dysentery bacillus. Certain of these cases have yielded evidence which might be indicative of the nature of the primary infection, but the difficulty of interpreting positive results, in so far as the mannite-fermenting bacilli are concerned, must be emphasized.

As Manson-Bahr has rightly indicated, all the points referred to above might usefully be re-investigated. Opportunity for this research exists in India, as it is probable that, notwithstanding the general impression to the contrary, bacillary dysentery is much more common amongst British troops in that country than the amoebic infection.

This communication, recording a few observations made during some years' laboratory experience of these problems, may be of interest to officers engaged in this work.