Further, it has already been pointed out that typhoid bacilli may be isolated from faeces by means of a series of doses of brilliant green and telluric acid, where direct plating, as well as the use of varying amounts of brilliant green alone, both fail to detect their presence (Browning, Mackie, and Smith). The following instance, which recently came under our observation, furnishes a fresh illustration of this latter highly important point. We are indebted to Dr. Pasteur for permission to use the clinical notes of the case.

Woman, aged 34. History of sixteen days' illness; pains in the head and abdomen, with slight abdominal tenderness, vertigo, and profound weakness. The faeces throughout have been firm and well formed. "Rose spots" present; also slight splenic enlargement (maximum recorded temperature 103.4° F.). Previous to the onset of illness the patient had for five weeks nursed a case said to be suffering from typhoid fever. Widal reaction (by the macroscopic method, using Dreyer's emulsion) shows marked agglutination of *B. typhosus* up to 1 in 200 serum dilution after two hours at 55° C. Specimen of faeces (solid, obtained by enema)—one loopful emulsified in 5 c.cm. normal saline; then one large loopful of emulsion added to each tube of medium (1 per cent. peptone water, neutral to litmus; two series—(a) containing brilliant green, and (b) brilliant green plus telluric acid).

10 c.cm. Peptone Water containing		Resulting Growth, after Incubation for 24 hours at 37° C., plated on MacConkey's Medium.
Telluric Acid 1 per cent.	Brilliant Green 1:10,000.	
0 ...	0.1 c.cm.	Coliform bacilli.
	0.2 "	" "
	0.35 "	" "
	0.5 "	Coliform bacilli which did not ferment inositol.
0.04 c.cm. in each tube	0.1 c.cm.	Coliform bacilli.
	0.2 "	" "
	0.35 "	" "
	0.5 "	<i>B. typhosus</i> in pure culture.

Plate of MacConkey's medium inoculated directly with the faeces showed nothing resembling *B. typhosus* present in more than 100 colonies.

The amount of faeces used was less than that commonly employed by us. As a rule, in the case of very fluid faeces a large loop (up to 1 in. diameter) is employed for inoculation; solid faeces are emulsified by rubbing up with several volumes of sterile water, and then one loopful is used to inoculate each tube.

The results obtained here show (1) that along with a negative result by direct culture on MacConkey's medium—the number of typhoid bacilli present in the faeces constituting probably much less than 1 per cent. of the total viable organisms—(2) no amount of brilliant green up to high doses enabled *B. typhosus* to flourish so as to permit it to be recovered in subcultures, but (3) the mixture of telluric acid and brilliant green yielded in this case a pure growth of *B. typhosus* in the tube containing the highest amount of green, whereas no typhoid bacilli were found in subcultures from any other tubes of the series. We shall not attempt to analyse the multiplicity of factors which may operate in such cases; but the practical issues are clear—namely, that (a) for the successful isolation of *B. typhosus* from faeces varying amounts of the differential reagents may be necessary, and (b) the use of telluric acid along with brilliant green may be essential in order to secure a positive result.

Summary.

1. In the examination of faeces for typhoid and paratyphoid bacilli, the value of a simple fluid medium containing brilliant green, in addition to direct plating, as a means of increasing the number of positive results, has been confirmed by a number of workers.

2. A single fixed concentration of brilliant green (0.5 c.cm. of green 1 to 10,000 per 10 c.cm. of peptone water) seems to leave little to be desired as a medium for the isolation of *B. paratyphosus* B; but this fixed medium does not yield so good results with *B. typhosus*, for which a series of doses of green are required in order to secure the optimum concentration, which depends on a number of variables.

3. In the examination of faeces for *B. typhosus* a series of varying amounts of brilliant green along with telluric acid may be essential in order to isolate this organism in cases where both direct plating and brilliant green alone fail.

* MacConkey's agar has been employed, since we have not found that, as prepared here, it compared disadvantageously with Endo's as a medium for the growth of *B. typhosus*.

4. The importance of detecting carriers for the prophylaxis of epidemics should justify the time spent in this extension of the "one-tube" method. Even in its unabbreviated form the procedure compares favourably, as regards consumption both of time and materials, with the old method of spreading a number of large plates directly with the faeces. The fact that the successful subcultures from the fluid medium frequently yield practically pure growths of the pathogenic organism is an important advantage of the method.

REFERENCES.

- Browning, Gilmour, and Mackie, *Journ. of Path. and Bact.*, vol. xviii, 1913, p. 146, and *Journ. of Hygiene*, vol. xiii, 1913, p. 335. Browning, Mackie, and Smith, *Journ. of Path. and Bact.*, vol. xix, 1914, p. 127. Browning and Thornton, *BRITISH MEDICAL JOURNAL*, August 14th, 1915. Clarke and Stokes, *Lancet*, March 11th, 1916. Dreyer, *Proc. Roy. Soc. Med.*, Medical Section, November 9th, 1915. Harvey, *R.A.M.C. Journ.*, 1915. Tidy, *Proc. Roy. Soc. Med.*, Medical Section, November 9th, 1915. Torres, *Journ. of Infect. Dis.*, vol. xiii, p. 263, 1913.

EXAMINATION OF FIFTY DYSENTERY CONVALESCENTS FOR CARRIERS.

BY

J. A. ARKWRIGHT, W. YORKE,
TEMPORARY CAPTAINS R.A.M.C.,

O. H. PRIESTLEY, AND W. GILMORE,
TEMPORARY LIEUTENANTS R.A.M.C.

The fifty men examined had all suffered from dysentery contracted on service, and with one exception gave a history of passing blood and slime for periods varying from three days to nine weeks. In four cases a history was also obtained of an illness resembling enteric fever. The convalescents were not selected, but were the first fifty examinations completed in the course of search for carriers in a convalescent camp.

The date of onset of the original illness ranged from May, 1915, to January, 1916, but all except two cases first showed symptoms of dysentery before the end of November, 1915. The examination began on February 16th, and ended on March 24th, 1916.

In every case, therefore, except two the illness began more than two months before the investigation here recorded. The examination of faeces was made in each case on three occasions, an interval of two or three, or occasionally more, days elapsing between the separate observations. The specimens of faeces reached the laboratory within half an hour of being passed. The examination consisted of: (1) Microscopic search for protozoa; (2) plating on MacConkey's neutral red bile-salt lactose agar, and subsequent examination of colourless colonies by means of carbohydrate media and agglutinating serums; (3) a specimen of blood was taken from each man on one occasion and its agglutinating power tested against stock emulsions.

Table showing the Results Obtained by Microscopic Examination and Culture.

<i>Entamoeba histolytica</i> was found in	9 men
<i>E. coli</i> was found in	25 "
<i>Lambia intestinalis</i> was found in	15 "
<i>B. dysenteriae</i> (Shiga) was found in	2 "
<i>B. dysenteriae</i> (Y) was found in	0 "
<i>B. typhosus</i> , <i>B. paratyphosus</i> A or B was found in	0 "
Negative results (neither protozoa nor bacteria)	14 "

Trichomonas intestinalis was noted in two cases, but was not looked for, especially on account of the need for rapid work. In all the cases in which *Entamoeba histolytica* was recognized tetragena cysts were observed, and large or small vegetative forms were also observed in some of these.

Further Analysis of the Results of Microscopic Examination.

<i>Entamoeba</i> was found alone in	6 men
<i>Entamoeba coli</i> was found alone in	12 "
<i>Lambia intestinalis</i> was found alone in	4 "
<i>E. histolytica</i> and <i>Lambia</i> were found in	1 man
<i>E. histolytica</i> and <i>E. coli</i> were found in	2 men
<i>E. histolytica</i> and <i>Trichomonas</i> were found in	1 man
Negative	14 men.

Total ... 50

In both cases from which *B. dysenteriae* (Shiga) was isolated *Entamoeba coli* was also found, and in one of them *Lambia* in addition. From the above statement it is seen that 11 (22 per cent.) men out of the 50 convalescents were found to be carriers of pathogenic micro-organisms (9 *E. histolytica*, 2 *B. dysenteriae*, Shiga). The specimens from the same man did not always yield the same result on each of the three examinations.

<i>E. histolytica</i> was found on 3 occasions in	... 2 men
" " " " 2 occasions in	... 6 "
" " " " 1 occasion in	... 1 man
<i>B. dysenteriae</i> (Shiga) was found on 2 occasions in	... 1 "
<i>B. dysenteriae</i> (Shiga) was found on 1 occasion in	... 1 "

A single examination in each case, therefore, would not have given so many positive results, and it is reasonable to suppose that more numerous examinations would have revealed additional carriers.

Results of Agglutination Tests with the Serum of Convalescents.

Agglutination tests were made with the serum of each man against *B. typhosus*, *B. paratyphosus* A and B, *B. dysenteriae* (Shiga and Y), and also against *M. melitensis*. Definite agglutination with a dilution of 1 in 100 was regarded as a positive result in the case of *B. typhosus* and *B. paratyphosus* A and B and *B. dysenteriae* (Y). A dilution of 1 in 50 was considered sufficient in the case of *B. dysenteriae* (Shiga), and 1 in 400 was the only dilution used with the *M. melitensis* emulsion.

The method used was macroscopic, and the reading was made after four hours at 37° C. and about eighteen hours at room temperature. All the men had been inoculated with *B. typhosus* vaccine, and in twenty-seven a positive agglutination reaction was obtained with *B. typhosus*. Seven had also received a mixed *B. paratyphosus* A and B vaccine. Agglutination of these bacilli was explicable by the inoculation in these cases, and the results have therefore been discarded.

In all the men negative agglutination results were obtained against *B. dysenteriae* (Y) and *M. melitensis*. A positive result was obtained with *B. dysenteriae* (Shiga) in twenty-one cases, including the two men from whom this bacillus was isolated. A positive result with *B. paratyphosus* B was obtained in four cases, but in no instance was a positive reaction obtained with *B. paratyphosus* A (leaving out of account the men who had received paratyphoid vaccine).

Multiple Infections.

Indications of multiple infection were given in six convalescents when the agglutination results were taken into consideration in addition to the microscopic and cultural results.

The serum of one man gave a positive reaction with *B. dysenteriae* (Shiga) and with *B. paratyphosus* B.

The serum of one carrier of *E. histolytica* gave a positive reaction with *B. dysenteriae* (Shiga) and with *B. paratyphosus* B.

The serum of three other carriers of *E. histolytica* gave positive reactions with *B. dysenteriae* (Shiga).

The serum of one other carrier of *E. histolytica* gave a positive reaction with *B. paratyphosus* B.

An attempt to diagnose the original illness or illnesses from which the convalescents had suffered by agglutination tests rests on much less secure ground than diagnoses based on microscopic cultural evidence. However, an opinion may be formed of the numerical relation of the different kinds of infection. From all the evidence obtained it appeared that:

4 men had suffered from infection with *E. histolytica* alone.

1 man had suffered from infection with *E. histolytica*, *B. dysenteriae* (Shiga), and *B. paratyphosus* B.

3 men had suffered from infection with *E. histolytica* and *B. dysenteriae* (Shiga).

1 man had suffered from infection with *B. dysenteriae* (Shiga) and *B. paratyphosus* B.

16 men (including 2 cases in which *B. dysenteriae* was isolated) had suffered from infection with *B. dysenteriae* (Shiga) alone.

For the remaining men no direct evidence was obtained as to the nature of the infection, but since they

gave negative agglutination results there is, perhaps, a presumption that the majority had been infected with *E. histolytica*.

Main Conclusion.

Among 50 dysentery convalescents examined, three to six months after the onset of their illness, 9 were still excreting *E. histolytica*, 2 were still excreting *B. dysenteriae* (Shiga). These 11 men were therefore potential sources for the outbreak of fresh epidemics.

AN EASY AND RAPID METHOD OF DOING WIDAL'S REACTION FOR TYPHOID.

BY

ALFRED C. COLES, M.D., D.Sc., F.R.S. EDIN.,
M.R.C.P. LOND.,

PHYSICIAN TO THE ROYAL NATIONAL SANATORIUM, BOURNEMOUTH.

The following is a rapid and simple method of determining, or at least of strongly suspecting, the presence or absence of typhoid in a non-inoculated person. It possesses the advantage of requiring very little blood, for with a few films such as are used for ordinary microscopic examination, we can (especially if typhoid is suggested by an absence of leucocytosis) perform the agglutinating test with one or two of these films.

1. With a colour grease glass writing pencil, or a piece of wax, draw a line across the middle of two slides at right angles to their long axes.

2. Spread a film of the blood to be examined on one half of each slide, and, when dry, spread a film of one's own blood, or that of a person who has not had typhoid or who has not been protectively inoculated against it, as a control, on the other half of each slide. The film may be as thin as that ordinarily used for stained films, or a little thicker. Dry.

3. By means of a platinum loop or pipette place a small drop of an emulsion of killed typhoid bacilli on the centre of each half of both slides, and with the platinum loop, or with a needle, rub the drop well over the film of blood, taking care not to pass from one half of the film to the other without sterilizing the needle.

4. On one slide carefully place a cover-glass on each half, taking care that the two cover-glasses are well separated in the middle by the mark of the grease pencil.

5. Place the other slide on a piece of wet blotting paper, and cover with a Petri dish to prevent evaporation for a period of fifteen to twenty minutes. At the end of that time dry carefully over the flame, and then stain.

Examine both halves of the first slide under a moderately low power. At the end of fifteen minutes (often much sooner) distinct clumps of agglutinated bacilli will be seen, provided the case be one of typhoid or the blood of one who has been prophylactically inoculated with a typhoid vaccine, whilst on the other half, the control, the bacilli show no signs of clumping. The film, stained with Leishman or Giemsa's stain, will show on one half patches of agglutinated bacilli, whilst on the control half the typhoid bacilli are more or less uniformly spread over the film. The red cells are practically invisible, but the leucocytes are clearly defined, and it is quite easy, provided the film has been thin, to determine the presence or absence of leucocytosis, and to make, if necessary, a differential count of the various forms of white cells present.

The emulsion I have been using is that made from a young agar culture of typhoid bacilli in saline solution, killed by the addition of 1 per cent. formalin. Emulsions of dead typhoid bacilli can be obtained from the Lister Institute.

I find the standard agglutinable cultures of Professor Dreyer as sent out from Oxford for the sedimentation method answer very well, especially if the emulsion be taken from the lower part of the bottle. Prophylactic typhoid vaccines do not give as clear results as emulsions from agar cultures, and the bacilli do not stain readily. Should the above give a positive result, a quantitative test may subsequently be made of the serum of the blood by Professor Dreyer's sedimentation method. Provided with an emulsion of killed paratyphoid A and paratyphoid B bacilli, the blood film may be tested against infection of these organisms in precisely the same way.

I am greatly indebted to Captain Alfred Friol, M.D., F.R.C.S.I., S.A.M.C., Bacteriologist to the South African General Hospital, for his kindness in confirming my results.