RECENT BACTERIOLOGICAL EXPERIENCES WITH TYPHOIDAL DISEASE AND DYSENTERY.

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WITH NOTES ON THE PROTOZOAN PARASITES IN THE EXCRETA.

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ONE result of the extension of hostilities to the Near East has been the opportunity afforded to the medical profession at home of studying on a fairly extensive scale certain infectious diseases met with only sporadically in time of peace. During the past two months (that is, since the middle of August, 1915) this hospital has admitted from the Dardanelles area a considerable number of cases presenting a recent history of acute dysentery with the classical symptoms of tenesmus and frequent evacuations of blood and mucus. In the great majority of these cases the stools on admission had either regained their normal character or were for a varying time after admission persistently loose, unformed, or semifluid. In a small minority of cases the disease at the time of admission still continued in a subacute or chronic form, with constant or intermittent discharge of blood and mucus in the dejecta. Simultaneously with these admissions of convalescent or subacute cases of dysentery there has been a succession of cases of typhoidal disease, in the main due to *B. paratyphosus A*. The bacteriological supervision of these cases, necessitating, on the one hand, the examination of numerous samples of excreta, and on the other, the carrying out of blood cultures and serological tests for purposes of diagnosis, has been no light addition to the ordinary routine of a bacteriological department attached to a hospital of 1,600 beds.

We venture to communicate the following notes on our bacteriological experiences, solely in the hope that they may be of interest and perchance of service to others working in similar fields and possibly under less advantageous surroundings.

EXAMINATION OF FAECES.

In the first place, it may be advisable to detail briefly our routine technique for the bacteriological examination of stools, urine, and blood, and for the performance of serological tests with the serums of patients.

When large numbers of stools have to be dealt with, the development of rapid and reliable methods of identification is of paramount importance, otherwise reports are apt to get in arrears, and confusion is likely to reign in the laboratory. Further, it is to the interest of the clinician that bacteriological diagnosis should be forthcoming at the earliest possible moment. The technique for faeces examinations here detailed is that to which we have been led by gradual modifications of the scheme with which we commenced operations, and it has proved very satisfactory.

ESSENTIALS.

1. MacConkey's bile-salt-lactose-neutral-red-agar.
2. Mannite peptone water containing Durham's tube.
3. Thoroughly tested specific agglutinating serums of high potency for *B. typhosus*, *B. paratyphosus A*, *B. paratyphosus B*, *B. enteritidis* Gaertner, *B. dysenteriae* Shiga, and *B. dysenteriae Y*. These serums we obtain from the Lister Institute.

The following dilutions of these serums are made with carbolyzed saline (0.5 per cent. carbolic in normal saline) and kept in bulk preferably but not necessarily in the cold chest.

1. *P.G. mixture* containing in equal parts, *Paratyphosus A* serum (1 in 66), *Paratyphosus B* serum (1 in 66), and *Gaertner* serum (1 in 66). Concentration of each in final mixture = 1 in 200.
2. *P.T. mixture* containing in equal parts, *Paratyphosus A* serum (1 in 66), *Paratyphosus B* serum (1 in 66), and typhoid serum (1 in 66). Concentration of each in final mixture = 1 in 200.

3. *Y serum* diluted 1 in 100, or 1 in 200.
4. *Shiga serum* diluted 1 in 100, or 1 in 200.
5. *Paratyphosus A serum* diluted 1 in 200.
6. *Paratyphosus B serum* diluted 1 in 200.
7. *Typhoid serum* diluted 1 in 200.
8. *Gaertner serum* diluted 1 in 200.

STEPS.

Portion of faeces emulsified in broth and allowed to stand on bench for an hour. One or two drops from the upper layers of the fluid are spread on the bile-salt-agar plates.

Next Morning.—Pick off several non-lactose colonies (discrete colonies wherever possible) and inoculate each colony into mannite and into ordinary broth. The number of colonies taken off will depend on many factors—for example, (1) the number of white colonies present, (2) the appearance of the colonies, (3) the clinical symptoms of the case if known, (4) the appearances of the stool.

Evening of Same Day.—After at least five hours' growth, examine the broth tubes, preferably with the dark ground microscope, and record motility or non-motility. At the same time inoculate an agar slope from each broth tube.

Second Morning.—The mannite tubes are examined and the results (acid and gas, acid only, or no change) recorded on the corresponding agar slopes.

1. If *acid and gas and motile*, test the corresponding slope with *P.G. mixture*. A loop of culture is emulsified in 4 or 5 c.cm. of saline. Equal parts of this emulsion and the *P.G. mixture* are placed in a small "agglutination" test tube and kept at 37° C. Examine the tube every hour and note if agglutination is taking place. If so, test the corresponding emulsion with *Paratyphosus A* serum, *Paratyphosus B* serum, and *Gaertner* serum separately (one dilution of each giving 1 in 400 in the final mixture). If agglutination takes place with *A* serum but not with *B* or *Gaertner* serum the emulsion may then be tested up to the full titre of the serum.

2. If *acid and gas and non-motile*, no further steps need be taken, but it must be remembered that a positive motility is final, while a negative result may leave a certain element of doubt, in which case it is wise to treat the culture as in 1. The extra labour is small.

3. *Acid only and motile*. Test with the *P.T. mixture*. (Many paratyphoid strains give little or no gas in the sugars they normally ferment, and for this reason it is necessary to treat all cultures giving acid only in mannite (and motile) as possible paratyphoids as well as typhoids.) If agglutination occurs, test further with *A*, *B*, and *T* serums separately.

4. *Acid only and non-motile*. Test with "Y" serum.

5. *No change on mannite and motile*. Discard for the time being, or, if time permits, test further to determine whether the culture is Morgan's No. 1 *Bacillus* or a member of the *Proteus* group.

6. *No change on mannite and non-motile*. Test with *Shiga* serum.

The emulsions are arranged in a test rack according as they have to be tested with *P.G. mixture*, *P.T. mixture*, *Y serum*, etc. Equal volumes of serum (say from the stock *P.G. mixture*) are placed in small "agglutination" tubes by aid of a large-bored capillary pipette with rubber test and fiducial mark, delivering about 0.4 c.cm.

Similar volumes of the corresponding emulsions are added by means of the same pipette, which is cleansed (between each operation) by boiling water from a beaker. As mentioned above, the tubes, after being placed in the incubator, should be examined every hour or so, and any that have reacted should be further tested if necessary. No negative results should be recorded, however, until the following morning, when the final readings are made.

NOTES WITH REGARD TO THE METHOD.

There has never been the slightest difficulty in differentiating *B. paratyphosus A*, *B. paratyphosus B*, and *B. Gaertner* by the specific serums. Absorption methods have never been necessary. As will be mentioned below, we have so far isolated only one member of the mannite-fermenting group of *B. dysenteriae* (Flexner-Y) which has agglutinated to the full titre of our "Y" serum. Organisms probably belonging to this group but inagglutinable by the specific serum are set aside for later examination at leisure. In view of the high specificity frequently met with in this group with respect to agglutination by specific serum, it is highly probable that we may be dealing with a group which can only be differentiated by a serum prepared by immunization with one or more of their number.

The *Shiga* strains we have obtained have all been agglutinated in high dilution by the specific *Shiga* serum (1 in 1,500 to 1 in 4,000). In the presence of decisive agglutination tests, confirmatory fermentation tests may with confidence be deferred to a more convenient time.

The following extract from our ledger is given to illustrate the method in practice:

Index No. 899.

Name. H.

Date. October 13th.

Particulars of Faeces.—Fairly stiff yellow stool. No blood or mucus seen.

Microscopic Examination.—No blood or mucus. No flagellates or amoebae.

MacConkey Plates.—A few suspicious colonies:

Colonies Picked Off:

A. Mannite + Motility + Discard.

B. Mannite A Motility + P.T. (++++), A 400 (++++), B. 400 (0), T. 400 (0).

C. Mannite A Motility + Reacted similarly to B.

B finally taken up to 1 in 3,200 (++++).

Report. *B. paratyphosus* A.

Technique for Blood Culture in Typhoidal Cases.

10 c.cm. blood from an arm vein are taken; 5 c.cm. are inoculated into a tube containing about 10 c.cm. of pure ox bile. The other 5 c.cm. are placed either in a flask of ordinary broth or in a mixture in equal parts of bile and broth.

The bile tube is examined next morning (hanging drop under dark ground microscope). If a motile organism is seen the bile is plated out on MacConkey's medium, or (preferably) an agar slope may be plentifully inoculated with the bile and a culture obtained for agglutination purposes next morning (or possibly the same evening). The bile tubes should be examined for at least four days, as we have on several occasions recovered *B. paratyphosus* A on the fourth day while apparently negative on the third. Before being finally discarded, all bile tubes should be plated out.

Ordinary broth has been most unsatisfactory. In many cases the broth has remained sterile while the bile tube has given positive results.

Technique for Widal Tests in Cases of Typhoid, Paratyphoid, and Dysentery.

The macroscopic method is used, small test tubes being required.

Standardized emulsions of *paratyphosus* A, *paratyphosus* B, *Gaertner*, *typhoid*, *Shiga*, etc., are made by emulsifying twenty-four-hour agar slopes in normal saline containing 1 in 1,000 formalin (except in the case of *paratyphosus* B, which is emulsified in half-normal saline containing formalin). These emulsions are standardized to a certain opacity corresponding to about 2,000 millions per c.cm. The tubes are placed in the incubator at 37° C. for at least two hours and then kept on the bench till next morning, when the final reading is taken and recorded. The reading is made in the first instance from the appearance of the sediment examined under a strong light and finally controlled by microscopical examination of the shaken-up mixture (the tube being rolled a definite number of times between the hands). A capillary tube is inserted into the highest dilution and a drop therefrom removed to a slide, then a drop from the next highest, and so on. The row of drops is then examined without any coverslip under the low power of the microscope.

CASES OF PARATYPHOID FEVER.

CASE 1.—O—y. Admitted August 23rd with fever which lasted till September 7th. *B. paratyphosus* A was recovered from the blood on August 26th.

Agglutination.—September 10th: r. A, 1 in 40 (++++), 1 in 80 (+), 1 in 160 (0); r. B, 1 in 40 (++++), 1 in 80 (0).

CASE 2.—E—s. Admitted August 26th with fever which lasted till September 17th. The patient had a relapse which lasted from October 2nd till October 14th. *B. paratyphosus* A recovered from the blood on August 27th.

Agglutination.—September 10th: r. A, 1 in 40 (++++), 1 in 80 (++++), 1 in 160 (++++), 1 in 320 (+), 1 in 640 (trace); r. B, 1 in 40 (0).

CASE 3.—W—s. Admitted August 23rd. Pyrexia on August 24th which lasted till September 14th. Relapsed September 18th. Temperature normal on October 1st. *B. paratyphosus* A recovered from the blood on August 31st.

Agglutination.—September 10th: r. A, 1 in 40 (0), 1 in 80 (0); r. A of Case 4, 1 in 40 (0), 1 in 80 (0); r. B, 1 in 40 (0).

CASE 4.—H—n. Admitted August 24th. Pyrexia from August 28th to October 6th; exacerbation from October 6th to October 29th. *B. paratyphosus* A was recovered from the blood on September 1st; and *B. dysenteriae* Shiga from the blood on October 11th. (See Case 14 in Dysentery series.)

Agglutination.—September 8th: r. A, 1 in 40 (++++), 1 in 80 (0), October 12th: r. A, 1 in 40 (++++), 1 in 80 (0), 1 in 160 (0); r. A (homologous), 1 in 40 (++++), 1 in 80 (trace), 1 in 160 (0); r. Shiga, 1 in 40 (trace), 1 in 80 (0); r. Shiga (homologous), 1 in 40 (0), 1 in 80 (0).

CASE 5.—O—a. Admitted August 27th with slight fever. Normal on September 17th; relapse September 19th to Sep-

tember 26th. *B. paratyphosus* A recovered from the blood on September 1st.

Agglutination.—September 10th: r. A, 1 in 40 (++++), 1 in 80 (+), 1 in 160 (0); r. A of Case 4, 1 in 40 (++++), 1 in 80 (+), 1 in 160 (0); r. B, 1 in 40 (0).

CASE 6.—C—s. Admitted August 29th. Pyrexia from September 1st to September 10th; since normal. *B. paratyphosus* A recovered from the blood on September 4th.

Agglutination.—September 10th: r. A, 1 in 40 (++++), 1 in 80 (0); r. B, 1 in 40 (++++). End-point not reached.

CASE 7.—W—n. Admitted August 23rd. Pyrexia from September 8th to 22nd. Since normal. *B. paratyphosus* A recovered from the blood on September 11th.

Agglutination.—September 11th: r. A, 1 in 40 (0), 1 in 80 (0); r. B, 1 in 40 (0), 1 in 80 (0).

CASE 8.—B—t. Admitted August 23rd. Pyrexia from September 11th to October 9th. *B. paratyphosus* A recovered from the blood on September 15th, and from the pus of a testicular abscess on October 21st in pure culture.

CASE 9.—F—t. Admitted September 16th with pyrexia which lasted till October 7th. *B. paratyphosus* A recovered from the blood on September 19th.

Agglutination.—September 27th: r. A, 1 in 40 (trace), 1 in 80 (0); r. B, 1 in 40 (0). October 5th: r. A, 1 in 40 (0); r. B, 1 in 40 (0); r. T, 1 in 40 (++++), 1 in 80 (++++), 1 in 160 (trace).

CASE 10.—A—r. Admitted September 16th. Pyrexia from September 21st to October 17th. *B. paratyphosus* A recovered from the blood on September 28th.

Agglutination.—October 9th: r. A, 1 in 40 (trace), 1 in 80 (0); r. B, 1 in 40 (0), 1 in 80 (0), 1 in 160 (++++), 1 in 320 (++++), 1 in 640 (trace). (Zone effect.)

CASE 11.—S—r. Admitted September 22nd. Pyrexia for six days before admission; normal on October 16th. *B. paratyphosus* A recovered from the blood on September 23rd, and from the faeces on the following day.

Agglutination.—September 27th: r. A, 1 in 40 (++++), 1 in 80 (++++), 1 in 160 (++++); end-point not reached; r. B, 1 in 40 (++++), 1 in 80 (0).

CASE 12.—T—y. Admitted September 10th. Pyrexia from September 18th to October 1st. *B. paratyphosus* B. recovered from the blood on September 23rd.

Agglutination.—September 27th: r. A, 1 in 40 (++++), 1 in 80 (++++), 1 in 160 (++++), 1 in 320 (0); r. B, 1 in 40 (++++), 1 in 80 (++++), 1 in 160 (++++); end-point not reached. October 9th: r. A, 1 in 40 (++++), 1 in 80 (0); r. B, 1 in 40 (++++), 1 in 80 (++++), 1 in 160 (++++), 1 in 320 (++++), 1 in 640 (++++), 1 in 1280 (trace); r. T, 1 in 40 (++++), 1 in 80 (++++), 1 in 160 (++++), 1 in 320 (++++), 1 in 640 (0).

CASE 13.—M—e. Admitted September 16th with fever which lasted till October 2nd; normal since. *B. paratyphosus* A recovered from the blood on September 21st.

Agglutination.—September 27th: r. A, 1 in 40 (0); r. B, 1 in 40 (0). October 5th: r. A, 1 in 40 (0); r. B, 1 in 40 (trace), 1 in 80 (0); r. T, 1 in 40 (++++), 1 in 80 (++++), 1 in 160 (++++), 1 in 320 (++++), 1 in 640 (++++), 1 in 1280 (0).

CASE 14.—M—y. Admitted September 5th with fever which lasted till September 21st. Severe relapse October 2nd to 27th. *B. paratyphosus* A recovered from the urine on October 6th, and from the blood on the following day.

CASE 15.—D—l. Admitted September 12th. Pyrexia September 22nd to October 7th. Slight relapse October 22nd to 26th. *B. paratyphosus* A recovered from the blood on October 1st.

CASE 16.—M—n. Admitted September 26th. Pyrexia from September 30th to October 21st. *B. paratyphosus* A recovered from the blood on October 3rd.

CASE 17.—M—n. Admitted September 13th with fever, which lasted till September 24th; since normal. Blood culture negative on September 15th. *B. paratyphosus* A recovered from faeces on September 14th.

Agglutination.—September 21st: r. A, 1 in 40 (++++), 1 in 80 (++++), 1 in 160 (++++); end-point not reached; r. B, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0); r. T, 1 in 40 (trace), 1 in 80 (0).

CASE 18.—F—y. Admitted September 6th. Pyrexia September 8th to 19th. *B. paratyphosus* A recovered from faeces on September 16th.

CASE 19.—R—n. Admitted September 26th. Pyrexia October 3rd to 17th. *B. paratyphosus* A recovered from faeces on September 28th.

CASE 20.—C—k. Admitted September 26th with pyrexia which lasted till October 7th. Blood culture negative on October 2nd. *B. paratyphosus* A recovered from faeces on September 28th.

Agglutination.—October 9th: r. A, 1 in 40 (++++), 1 in 80 (trace), 1 in 160 (0); r. B, 1 in 40 (0); r. T, 1 in 40 (++++), 1 in 80 (++++), 1 in 160 (trace), 1 in 320 (0).

CASE 21.—H.—J. Admitted September 9th. Pyrexia September 20th to October 13th. *B. paratyphosus* B recovered from faeces on September 25th.

Agglutination.—October 3th: v. A, 1 in 40 (++), 1 in 80 (+), 1 in 160 (0); v. B, 1 in 40 (0), 1 in 80 (+++), 1 in 160 (+++), 1 in 320 (++), 1 in 640 (++), 1 in 1280 (trace). Note initial inhibition zone.

CASE 22.—G.—n. Admitted September 16th. Had dysentery in August with blood and mucus. Slight diarrhoea on admission, with fever which lasted till September 27th. *B. paratyphosus* A recovered from faeces on September 23rd.

Agglutination.—September 27th: v. A, 1 in 40 (0), 1 in 80 (0); v. B, 1 in 40 (0), 1 in 80 (0); v. Shiga, 1 in 40 (+++), 1 in 80 (++), 1 in 160 (+), 1 in 320 (0).

CASE 23.—H.—n. Admitted September 16th. Pyrexia September 18th to October 13th. Blood culture negative on September 25th. *B. paratyphosus* A recovered from faeces on October 13th and 15th.

Agglutination.—October 9th: v. A, 1 in 40 (+), 1 in 80 (+), 1 in 160 (0); v. B, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0); v. T., 1 in 40 (+) 1 in 80 (+), 1 in 160 (trace), 1 in 320 (0).

CASE 24.—C.—n. Admitted September 9th. Pyrexia September 12th to 20th; relapse September 30th to October 22nd. Blood culture negative on September 18th. *B. paratyphosus* A recovered from faeces on October 11th.

Agglutination.—October 5th: v. A, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0); v. B, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0).

In the following the diagnosis rests so far on serological data.

CASE 25.—N.—h. Admitted August 23rd with fever which lasted till September 8th; relapse September 17th to October 3rd. Blood culture negative on August 26th.

Agglutination.—September 10th: v. A, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+), 1 in 320 (0); v. B, 1 in 40 (0), 1 in 80 (0).

CASE 26.—C.—e. Admitted August 23rd with fever; normal September 1st; relapse September 23rd to October 6th. Blood culture negative on August 26th.

Agglutination.—September 8th: v. A, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+++); limit not reached; v. B, 1 in 40 (+), 1 in 80 (0).

CASE 27.—B.—r. Admitted August 27th with fever which became normal on September 13th. Pyrexia again from September 14th to 18th; since normal. Blood culture negative on August 31st.

Agglutination.—September 10th: v. A, 1 in 40 (++), 1 in 80 (++), 1 in 160 (++); limit not reached; v. A of Case 4, 1 in 40 (+), 1 in 80 (0); v. B, 1 in 40 (0), 1 in 80 (0).

CASE 28.—B.—t. Admitted August 30th with fever which lasted till September 20th. Blood culture negative on September 1st.

Agglutination.—September 1st: v. A, 1 in 40 (++), 1 in 80 (+++), 1 in 160 (+), 1 in 320 (0). September 10th: v. A, 1 in 40 (+++), 1 in 80 (+), 1 in 160 (0); v. B, 1 in 40 (0), 1 in 80 (0).

CASE 29.—G.—m. Admitted August 30th with acute fever which lasted till September 10th; since normal. Blood culture negative on September 2nd.

Agglutination.—September 10th: v. A, 1 in 40 (++), 1 in 80 (++), 1 in 160 (0); v. B, 1 in 40 (0), 1 in 80 (0).

CASE 30.—F.—r. Admitted August 8th with pyrexia which lasted till September 1st. Blood culture negative on August 10th.

Agglutination.—September 5th: v. A, 1 in 100 (+++). Not taken further.

CASE 31.—P.—r. Admitted August 30th with slight fever (convalescent case). Blood culture negative on September 6th.

Agglutination.—September 10th: v. A, 1 in 40 (++), 1 in 80 (+), 1 in 160 (0); v. B, 1 in 40 (0), 1 in 80 (0).

CASE 32.—J.—s. Admitted August 23rd with fever which lasted till September 1st; since normal.

Agglutination.—September 7th: v. A, 1 in 40 (+++), 1 in 80 (+), 1 in 160 (0); v. B, 1 in 40 (0), 1 in 80 (0).

CASE 33.—G.—m. Admitted September 3rd with slight fever (convalescent case). Blood culture negative on September 7th.

Agglutination.—September 10th: v. A, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (++); limit not reached; v. B, 1 in 40 (+), 1 in 80 (0).

CASE 34.—N.—s. Admitted August 26th with fever complicated by wound. Blood culture negative on September 7th.

Agglutination.—September 10th: v. A, 1 in 40 (+++), 1 in 80 (+), 1 in 160 (0); v. B, 1 in 40 (0), 1 in 80 (0).

CASE 35.—F.—r. Admitted September 3rd with fever; nine days of fever before admission; fever lasted till September 30th. Blood culture negative on September 8th.

Agglutination.—September 9th: v. A, 1 in 40 (++), 1 in 80 (++), 1 in 160 (0).

CASE 36.—H.—s. Admitted September 9th with very slight pyrexia which continued till September 17th; history of fever previous to admission. Blood culture negative on September 11th.

Agglutination.—September 11th: v. A, 1 in 40 (trace), 1 in 80 (0), 1 in 160 (0); v. B, 1 in 40 (+++), 1 in 80 (++), 1 in 160 (+), 1 in 320 (0).

CASE 37.—V.—e. Admitted August 30th. Had dysentery, with blood and mucus, in Gallipoli. Blood culture negative on September 2nd.

Agglutination.—September 10th: v. A, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0); v. B, 1 in 40 (+++); not taken further. September 14th: v. B, 1 in 40 (++), 1 in 80 (+), 1 in 160 (+), 1 in 320 (0).

CASE 38.—K.—y. Admitted September 10th with slight fever (convalescent case). Blood culture negative on September 15th.

Agglutination.—September 21st: v. A, 1 in 40 (++), 1 in 80 (+++), 1 in 160 (+), 1 in 320 (0); v. B, 1 in 40 (+++), 1 in 80 (++), 1 in 160 (++); not taken further.

CASE 39.—P.—n. Admitted September 10th with very slight pyrexia (convalescent case). Blood culture negative on September 15th.

Agglutination.—September 21st: v. A, 1 in 40 (trace), 1 in 80 (0), 1 in 160 (0); v. B, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (++); not taken further.

CASE 40.—R.—n. Admitted September 10th. No high temperature (convalescent case). Blood culture negative on September 15th.

Agglutination.—September 21st: v. A, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0); v. B, 1 in 40 (+++), 1 in 80 (+), 1 in 160 (+), 1 in 320 (0); v. T., 1 in 40 (+), 1 in 80 (+).

CASE 41.—M.—e. Admitted September 5th. Pyrexia from September 8th to September 28th. History of a previous fever in Malta. Blood culture negative on September 15th.

Agglutination.—September 21st: v. A, 1 in 40 (++), 1 in 80 (+), 1 in 160 (0); v. B, 1 in 40 (0), 1 in 80 (0).

CASE 42.—L.—t. Admitted September 9th with fever which continued till September 23rd.

Agglutination.—September 16th: v. A, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0); v. B, 1 in 40 (+++), 1 in 80 (++), 1 in 160 (++); limit not reached.

CASE 43.—W.—d. Admitted September 10th. Pyrexia September 11th to 23rd. Blood culture negative on September 16th.

Agglutination.—September 21st: v. A, 1 in 40 (trace), 1 in 80 (0); v. B, 1 in 40 (++), 1 in 80 (++), 1 in 160 (+), 1 in 320 (0); v. T., 1 in 40 (+), 1 in 80 (0), 1 in 160 (0).

CASE 44.—P.—r. Admitted September 10th with fever which lasted till October 11th. Relapse October 12th to 19th. Blood culture negative on September 17th.

Agglutination.—September 21st: v. A, 1 in 40 (++), 1 in 80 (+++), 1 in 160 (+), 1 in 320 (0); v. B, 1 in 40 (++), 1 in 80 (+), 1 in 160 (+), 1 in 320 (0).

CASE 45.—F.—r. Admitted August 28th. Pyrexia from September 9th to 26th.

Agglutination.—September 21st: v. A, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (0); v. B, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0).

CASE 46.—M.—r. Admitted September 16th with fever which lasted till October 5th. Blood culture negative on September 16th.

Agglutination.—September 21st: v. A, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (++); limit not reached; v. B, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (0); v. T., 1 in 40 (+), 1 in 80 (+). October 5th: v. A, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+), 1 in 320 (trace), 1 in 640 (0); v. B, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (++), 1 in 320 (++), 1 in 640 (trace), 1 in 1280 (0).

CASE 47.—D.—n. Admitted August 20th. Pyrexia September 10th to 19th; normal since. Blood culture negative on September 13th.

Agglutination.—September 21st: v. A, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+); limit not reached; v. B, 1 in 40 (+), 1 in 80 (0), 1 in 160 (0).

CASE 48.—S.—r. Admitted September 16th with slight irregular fever, which lasted till October 5th.

Agglutination.—October 1st: v. A, 1 in 40 (++), 1 in 80 (+), 1 in 160 (trace); v. B, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0); v. T., 1 in 40 (+), 1 in 80 (++), 1 in 160 (++), 1 in 320 (trace), 1 in 640 (0).

CASE 49.—C.—s. Admitted September 26th with pyrexia, which lasted till October 20th. Blood culture negative on October 2nd.

Agglutination.—October 3rd: v. A, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0); v. B, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (++), 1 in 320 (++), 1 in 640 (0); v. T., 1 in 40 (+++), 1 in 80 (++), 1 in 160 (++), 1 in 320 (trace), 1 in 640 (0).

CASE 50.—G.—s. Admitted September 16th. Pyrexia September 25th to October 11th; since normal. Blood culture

negative on October 6th. *B. dysenteriae* Shiga isolated from faeces on October 18th.

Agglutination.—October 13th: r. A, 1 in 40 (0), 1 in 80 (0); v. B, 1 in 40 (+++), 1 in 80 (++), 1 in 160 (0). October 22nd: r. A, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0); v. B, 1 in 40 (trace), 1 in 80 (0), 1 in 160 (0); r. Shiga, 1 in 40 (+), 1 in 80 (0), 1 in 160 (0).

CASES OF ENTERIC FEVER.

CASE 1.—R—s. Admitted September 16th with pyrexia, which continued till October 21st. *B. typhosus* recovered from the blood on September 16th.

Agglutination.—September 27th: r. A, 1 in 40 (+), 1 in 80 (0), 1 in 160 (0); v. B, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (0); r. T., 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+++), 1 in 320 (+++), 1 in 640 (+++), 1 in 1280 (+), 1 in 2560 (0). October 5th: 1 in 40 (0), 1 in 80 (0); v. B, 1 in 40 (0), 1 in 80 (0); r. T., 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+++), 1 in 320 (+++), 1 in 640 (+++), 1 in 1280 (+), 1 in 2560 (0).

CASE 2.—M—d. Admitted September 11th. Pyrexia September 11th to 17th; normal September 18th to 22nd; very acute fever September 22nd to October 10th; normal since. *B. typhosus* recovered from the blood on September 25th.

CASE 3.—M—d. Admitted August 30th with symptoms pointing to ulcerative endocarditis. *B. typhosus* and streptococci recovered from the blood on September 2nd. Death took place on September 6th. At the autopsy the Peyer's plaques were congested and inflamed, but no actual ulceration was found in the gut. The mesenteric glands were enlarged and acutely congested. The spleen weighed 12½ oz., and was soft and diffident. The gall bladder was healthy; there were no calculi. Left ventricle greatly hypertrophied. Cusps of aortic valve united together from old endocarditis. No recent vegetations. There was also a discharging wound on the left shoulder.

REMARKS WITH REGARD TO THE CASES RECORDED ABOVE.

It may be well to point out, in the first instance, that diagnosis only has been our object—in fact, our only possible object. When the blood culture was positive no further work was necessary, but in a certain number of cases we have made careful serological tests in order to obtain some estimate of the reliability of the latter for diagnostic purposes. As a rule the agglutination test was not performed until some days or perhaps a week (or more) had elapsed after the positive or negative blood culture.

Of the first group of 24 cases in which the causative bacillus was recovered from the blood, faeces, or urine, it will be observed that 8 or 9 cases gave little or no agglutination with the corresponding organism. The remainder, however, gave as a rule very definite reactions with the organism found to be the infecting agent.

Titres so high as 1 in 320 have been obtained in paratyphoid A infections, but a definite reaction even in 1 in 40 is to be regarded as significant in these A infections. Normal serums in our experience have given no reaction with *B. paratyphosus* A in this dilution.

In paratyphoid A cases also the serum has rarely given a group reaction with paratyphoid B. On the other hand, in the few paratyphoid A cases in which the typhoid Widal has been tested (Nos. 9, 13, 17, 20, 23) this has proved to be very low except in one case (No. 13), where agglutination of *B. typhosus* was obtained up to 1 in 640 dilution. (All cases, so far as could be ascertained, had been inoculated against enteric fever.)

B. paratyphosus B was recovered from two cases only (Nos. 12, 21). In case No. 12 the serum was tested on two occasions, when very high titres with paratyphoid B were found. There was also—at least on the first occasion—a very definite group reaction with *B. paratyphosus* A, which, however, had apparently in great part disappeared before the second test. This phenomenon may be analogous to that noted recently by certain Continental workers who have recorded positive typhoid Widal's in the early stages of bacillary dysentery. As the dysentery Widal became established the group reaction tended to disappear. With regard to the second group of cases (25 to 50), in which the diagnosis has rested on serological data, 15 (namely, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 41, 45, 47, 48) were almost certainly cases of paratyphoid A fever, 8 (namely, 36, 37, 39, 40, 42, 43, 49, 50) almost certainly paratyphoid B fever, while 3 (namely, 38, 44, 46) were either A or B, or mixed infections. In this latter group of three only the recovery of the actual causative agent or agents would effectively clear up the diagnosis. Apparently, however, many of these cases came in during the convalescent stage, when blood culture

was not likely to yield positive results. In 5 cases (namely, 40, 43, 46, 48, 49) the typhoid Widal was tested, but only in 2 (namely, 48, 49) was a well-marked positive reaction evident. Three cases only of enteric fever have occurred, *B. typhosus* being recovered from the blood in all. Case No. 3, which came to autopsy, has been already referred to. In case No. 1 the serum was carefully titrated against paratyphoid A, paratyphoid B, and *B. typhosus* on two occasions. A remarkably strong typhoid reaction was obtained on the first occasion with distinct evidence of group reaction with A and B (especially the latter). On the second occasion this group reaction had completely disappeared, while the specific typhoid Widal remained at the same high level.

On the whole we are strongly of opinion that the serological test in typhoid disease, while of the greatest service in practical diagnosis, should be employed only as a second string when bacteriological methods have failed to reveal the causative agent. To this object, namely, the isolation of the causative bacillus from the blood or excreta, chief attention should be directed. For statistical purposes, there can be no doubt that the best evidence is that derived from positive bacteriological findings.

No doubt the value of the serological test, and more particularly the interpretation of the same in doubtful cases, might be greatly improved by repeated titration of the serum from the commencement of the disease onwards, but a routine of this scope does not come within the realm of practical diagnosis, and moreover may fail entirely to detect the real nature of pyrexias during which (possibly because they are of short duration and small intensity) agglutinin development in measurable or significant degree fails to take place. A more detailed analysis of the results is for the moment deferred.

Mixed Infections.

The question of mixed infections, or rather of secondary infections, occurring during the convalescent or possibly carrier stage of the first infection, demands notice. Most of the paratyphoid cases have come in with a history of dysentery. Where typhoid disease and dysentery are simultaneously prevalent, the occurrence of post-dysenteric paratyphoid fever or post-paratyphoid dysentery is to be expected.

In Case 22, which gave a history of dysentery, *B. paratyphosus* A was recovered from the faeces. The patient's serum did not agglutinate A or B, but gave a very marked reaction with the Shiga bacillus. In Case 50 also, although the serum gave a significant agglutination with paratyphoid B, the organisms recovered from the stools were *B. dysenteriae* Shiga and *B. enteritidis* Gaertner.

Case 4, in which Shiga was obtained from the blood during a so-called relapse in the course of a definitely diagnosed paratyphoid A fever, was probably also one of post-dysenteric paratyphoid in a person who had become a dysentery (Shiga) carrier. From another case—not listed—of convalescent dysentery whose serum agglutinated Shiga in high dilution, *B. paratyphosus* A has recently been recovered in the faeces.

Cases of this kind will doubtless tend to increase, and they demand careful bacteriological attention, both from the point of view of science generally and preventive medicine in particular. In the absence of further data it is impossible to discuss the matter further at this stage.

BACILLARY DYSENTERY.

A large number of convalescent cases of dysentery have arrived with few or no symptoms. The stools were examined, as a rule, only once (and with generally negative results), and the patients were not retained in hospital. Among these admissions, however, were a few subacute or chronic cases either still passing blood and mucus or soft fluid unformed motions with or without mucus.

From 15 cases of this kind *B. dysenteriae* Shiga has been recovered. (See list of cases below, with notes regarding the appearance of the motions.) Agglutination tests have been performed in most of these cases with the stock Shiga strain and occasionally with the homologous organism also.

Apparently, during convalescence, very high titres may be expected.

One case only of Flexner's dysentery occurred, the

infection running its course from start to finish in the hospital. It lasted only a few days, and was heralded by high fever and the passage of blood and mucus.

As mentioned above, samples of stools have been examined, from which organisms probably belonging to the mannite-fermenting group of *B. dysenteriae* have been recovered, which do not agglutinate with the specific Y-serum available. The only case of dysentery which ended fatally soon after admission, and which showed intense enteritis of the large bowel at autopsy, was probably due to infection by a member of this group. From the bloody and mucoid stool before death, and also from the colon at autopsy, organisms of this type were recovered. Their consideration must remain over for the present.

The following list of cases, with particulars of the nature of the stools and the organisms isolated, is appended.

CASES OF BACILLARY DYSENTERY.

(Subacute or Chronic and Convalescent Cases.)

CASE 1.—L.—t. Admitted July 27th. Diarrhoea eight weeks before admission. No history of blood and mucus before admission. Mucoid stool of July 27th showed microscopically: Blood, pus, catarrhal cells, *Lambia intestinalis* (inactive), and cysts of the same; *B. dysenteriae* Shiga recovered in almost pure culture. Two other samples on the same day had similar features, except that there was much less pus and blood and far more food detritus, muscle fibres, and innumerable bacteria.

A sample taken on July 29th was apparently pure mucus. Microscopically: Numerous flagellate cysts were found. *B. dysenteriae* Shiga again recovered.

Sample of July 30th: Quite different from previous samples. Soft creamy stool not containing blood or pus, but numerous actively motile flagellates (*Lambia* and *Macrostoma*).

July 31st: Pure mucus stool. No flagellates seen. Some *Lambia* cysts present.

August 1st: Mucus stool, with pus and little blood. No active flagellates or cysts seen. *B. dysenteriae* Shiga recovered.

August 2nd: Greenish grumous stool, containing undigested muscle fibres, very few pus cells, and innumerable bacteria. No active flagellates.

August 3rd: Greenish-yellow mucoid stool. No motile flagellates seen. *Macrostoma* cysts present.

August 4th: Creamy stool like that of July 30th. Swarming with flagellates.

From the grumous and creamy stools, which contained food residues and many bacteria, *B. dysenteriae* Shiga could not be recovered.

Blood count on August 2nd:

Red cells	...	...	4,350,000
White cells	...	...	16,000
Differential count:			
Small lymphocytes	...	...	3.7 per cent.
Large lymphocytes	...	...	9.4 "
Large mononuclears	...	...	8.1 "
Plasma cells	...	...	1.3 "
Polymorphonuclears	...	...	76.9 "
Eosinophiles	...	...	0.3 "

Agglutination.—October 18th: r. Shiga, 1 in 40 (++), 1 in 80 (+), 1 in 160 (0); v. Shiga (homologous), 1 in 40 (+), 1 in 80 (trace), 1 in 160 (0).

The serum of the patient gave only a trace of agglutination with the homologous Shiga in 1 in 10 (performed July 29th).

This patient is still in hospital (October 28th), and is slowly putting on weight after a period of considerable emaciation.

CASE 2.—S.—r. Admitted August 23rd. Dysentery started with blood and mucus three months before admission.

First sample of August 24th: Like fluid blood with mucus. No flagellates present. *B. dysenteriae* Shiga recovered.

Second sample of same day: Fluid brownish grumous material containing numerous flagellates. *B. dysenteriae* Shiga not recovered. *B. paratyphosus* B recovered.

August 25th: Stool containing blood, mucus, and pus, but no flagellates. *B. dysenteriae* Shiga recovered.

Agglutination.—September 23rd: v. Shiga, 1 in 20 (+++), 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+++); not taken further; v. B, 1 in 40 (+), 1 in 80 (trace), 1 in 160 (0). October 18th: v. Shiga, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+++), 1 in 320 (+++), 1 in 640 (++), 1 in 1280 (+), 1 in 2560 (0); v. Shiga (homologous), 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+++), 1 in 320 (+), 1 in 640 (0).

This patient is slowly convalescing in hospital.

CASE 3.—D.—t. Admitted September 16th. Diarrhoea started on August 22nd. Blood appeared in the dejecta eight days after commencement of illness. The acute symptoms were over about September 11th. On September 18th the patient passed a watery stool containing mucus, but no flagellates or amoebae. *B. dysenteriae* Shiga recovered.

Agglutination.—September 23rd: v. Shiga, 1 in 20 (+++), 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+), 1 in 320 (0).

CASE 4.—W.—e. Admitted September 9th with slight diarrhoea and some abdominal pain. Dysentery apparently began about June 10th. On September 14th the patient passed a

formed normal solid stool. *B. dysenteriae* Shiga recovered. Probably carrier case.

Agglutination.—September 23rd: v. Shiga, 1 in 20 (+++), 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+++). Not carried further.

CASE 5.—R.—s. Admitted September 27th. Dysentery with blood and mucus started at the end of August. Slight diarrhoea present on admission. On September 18th passed a yellowish semifluid stool containing some mucus. No flagellates or amoebae seen. *B. dysenteriae* Shiga recovered.

Agglutination.—September 23rd: v. Shiga, 1 in 20 (0), 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+++). Not taken further. Note initial inhibition zone.

CASE 6.—F.—b. Admitted September 13th. Dysentery commenced on August 10th, with blood and mucus. He has frequently passed blood since admission. Subacute case. Sample of September 22nd: Semifluid, brownish-yellow stool with a little mucus. No flagellates or amoebae. *B. dysenteriae* Shiga recovered.

Agglutination.—September 23rd: v. Shiga, 1 in 20 (+++), 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (trace).

CASE 7.—P.—k. Admitted September 26th. Sick for a month previous to admission. Diarrhoea still present on admission and some abdominal pain. Sample of September 28th: Greenish-yellow mucoid stool. No flagellates present, but some amoebic cysts (probably *E. coli*). *B. dysenteriae* Shiga recovered.

Agglutination.—October 12th: v. Shiga, 1 in 40 (+++), 1 in 80 (+), 1 in 160 (0); v. Y, 1 in 40 (+++), 1 in 80 (+), 1 in 160 (0).

CASE 8.—W.—h. Admitted September 13th. Dysentery began in hospital on October 1st; ten motions on that day, with blood and mucus, and a temperature of 104°; watery diarrhoea for a few days afterwards. Acute dysentery with blood and mucus, lasting only a few days. Sample of October 2nd: Fluid stool with much blood and mucus and pus. No flagellates. *B. dysenteriae* Y recovered.

Agglutination.—October 12th: v. Shiga, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0); v. Y, 1 in 40 (+), 1 in 80 (0).

CASE 9.—W.—e. Admitted September 26th. Dysentery began at the end of August with passage of blood and mucus. Some diarrhoea and abdominal pain still present on September 30th. Convalescent case. Sample of September 30th: Brownish-yellow loose stool. No flagellates or amoebae. *B. dysenteriae* Shiga recovered.

Agglutination.—October 12th: v. Shiga, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (0); v. Y, 1 in 40 (+++), 1 in 80 (+), 1 in 160 (0).

CASE 10.—Admitted September 23rd. Diarrhoea for two months previously. Blood and slime appeared in the motions about September 12th. Convalescent case. Stools occasionally loose, and slight abdominal pain. Sample of September 24th: Liquid pea-soup character containing mucus. No flagellates, amoebae, or blood. *B. dysenteriae* Shiga recovered.

Agglutination.—October 12th: v. Shiga, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0); v. Y, 1 in 40 (+), 1 in 80 (0).

CASE 11.—M.—w. Admitted October 11th. Dysentery with passage of blood and mucus commenced on August 12th. Diarrhoea with mucus still present on admission. Convalescent case. Sample of October 11th: Loose yellow mucoid stool. No blood or pus. Some cysts of *E. coli*. *B. dysenteriae* Shiga recovered.

Agglutination.—October 18th: v. Shiga, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+++), 1 in 320 (trace), 1 in 640 (0); v. Shiga (homologous), 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (trace), 1 in 320 (0).

CASE 12.—B.—y. Admitted September 26th. Dysentery began on August 16th. Convalescent case. Sample of October 20th: Stiff dark brown stool. No blood or mucus. No active flagellates, but a few (?) *Macrostoma* cysts. *B. dysenteriae* Shiga recovered.

Agglutination.—October 22nd: v. Shiga, 1 in 40 (+++), 1 in 80 (+++), 1 in 160 (+), 1 in 320 (0); v. B, 1 in 40 (+++), 1 in 80 (+), 1 in 160 (0); v. A, 1 in 40 (0), 1 in 80 (0), 1 in 160 (0).

CASE 13.—G.—s. Convalescent case (see No. 50 of Paratyphoid series). Sample of October 18th: Loose yellow slightly mucoid stool. No blood, but numerous *Lambia* cysts. *B. dysenteriae* Shiga recovered, also *B. enteritidis* Gaertner.

CASE 14.—H.—n. (See No. 4 of Paratyphoid series.) *B. paratyphosus* A was recovered from the blood of this case on September 1st (about the fifth day of pyrexia). An exacerbation of pyrexia (so-called relapse) occurred on October 6th, and on October 11th a blood culture yielded *B. dysenteriae* Shiga in pure culture. This case was, in all probability, one of post-dysenteric paratyphoid A fever.

CASE 15.—D.—y. Admitted October 8th. Dysentery with blood and mucus began at the end of May. Slight diarrhoea since admission. Sample of October 22nd: Loose yellow stool containing mucus and pus, but no blood. *B. dysenteriae* Shiga recovered, also *B. enteritidis* Gaertner.

REMARKS ON CASES REPORTED ABOVE.

It will be noted that the stool from which the specific organisms were recovered, was not invariably of the typical mucoid variety with blood. In several cases the stool was of the watery order, with or without admixture of mucus, and in two cases at least the stool was normal and formed. This point is, of course, by no means new, but it has a distinct bearing on the preventive medicine and the carrier question in relation to bacillary dysentery. This, however, we do not propose to discuss here for lack of relevant data. The general carrier question in relation to bacillary dysentery is fully discussed elsewhere.¹ All that we can definitely say at present with regard to the carrier question in relation to our cases is that the specific organism has been isolated at periods varying from one to four months after the onset of the infection. Case No. 14, in which *B. dysenteriae* Shiga was recovered from the blood during a pyrexial exacerbation in the course of paratyphoid A fever, deserves notice. So far as we can ascertain, this finding is unique. *B. dysenteriae* "Y" has recently been recovered from the blood during life in a series of cases with obscure clinical picture (partly typhoid-like, partly dysentery-like) by Ghon and Roman,² and the explanation suggested by these authors was that the cases were really dysentery carriers secondarily infected with enteric.

The dysentery organism had succeeded in invading the blood stream, perhaps owing to the disordered condition of the bowel. In our case there is little doubt that the patient was a dysentery carrier secondarily infected by *B. paratyphosus* A. The patient had suffered severely from diarrhoea previous to his attack of paratyphoid fever. During the pyrexial exacerbation there was no return of blood and mucus in the dejecta.

No cases of amoebic dysentery have been met with (see section on flagellates, etc.). Several patients have been admitted with a history of having had injections of emetine. In no case, however, have motile forms or cysts of *E. tetragena* been detected.

The question of flagellate dysentery is discussed in the next section.

To the resident medical officers of the hospital we are greatly indebted for their cordial co-operation.

INTESTINAL PROTOZOAN PARASITES PRESENT IN THE FAECES.

Up to the time of writing, protozoan parasites have been found in the faeces of 26 cases. In several instances more than one kind of parasite occurred at the same time. The following is a list of the parasites observed:

Parasite.	No. of Cases.
<i>Lamblia intestinalis</i> (active and (or) encysted) ...	7
<i>Macrostoma mesnili</i> (active) ...	6
<i>Trichomonas hominis</i> (active) ...	3
<i>Cercomonas hominis</i> (active and encysted) ...	1
Active flagellates, not specifically determined (probably <i>Macrostoma</i> or <i>Trichomonas</i>) ...	4
Coccidian cysts ...	5
<i>Entamoeba coli</i> cysts ...	6
<i>Blastocystis hominis</i> ...	5

As there are very few records in England of these human intestinal parasites, it may be of service to give a

few notes with regard to them, particularly as they are likely to be met with in the examination of faeces in cases of dysentery or diarrhoea among soldiers home from the Dardanelles or France.

The examination is best made by taking up a very small quantity of the faeces on a platinum loop and mixing it well with a drop of sterile saline (0.5 per cent.), sufficient in amount to run under a cover-slip. The faeces must be well diluted, otherwise cysts especially are apt to be overlooked. The faeces should be examined as freshly as possible, because after four or five hours most of the active flagellates become motionless, degenerate and die. If the sample is kept in an incubator, at blood temperature, a few individuals will usually remain sluggishly motile for some hours longer. A convenient and rapid way of making a permanent preparation is as follows: A thin smear of the faeces, diluted as above, is made in the same manner as an ordinary blood film. The slide is immediately placed in a stain tube containing at the bottom a small quantity of 4 per cent. osmic acid solution + a drop of glacial acetic for fixation, and is left in for about ten seconds. Allow the smear to dry in air and then put in absolute alcohol for a quarter of an hour to harden.

Wash with tap water and stain with Giemsa (1 drop to 1 c.c.m.) for twenty minutes or so. Rinse with tap water. The smear must be fixed before drying, otherwise the active flagellates, which are soft and plastic, will be round and flattened, and usually unrecognizable as such.

Lamblia intestinalis (Fig. 1, A-D).—The active individual has a characteristic and unmistakable appearance. In shape it is like a flattened pear, with a broad, rounded anterior end and a tapering

hinder extremity. As is well known, there is a large saucer-like depression or concavity on the ventral side, in the anterior part of the body, by which the creature applies itself to the intestinal cells. This depression is best seen when the flagellate turns over on its side in moving. The creature does not change in shape at all—that is, it is not amoeboid. In stained preparations the double arrangement of the organelles is seen—two nuclei, four pairs of flagella, and two axial rods (axostyles). In Fig. 1, B, the karyosomes of the nuclei have come out well, but in Fig. 1, A, the nuclei have stained uniformly, as generally happens with Giemsa, though all these flagellates have karyosomatic nuclei. The cysts are frequently present in the stools when no active forms can be found. They are elongate-ovoid and slightly smaller than the active forms. Even in life four nuclei can generally be made out; also, in early cysts, remains of the flagella and axostyles. In stained preparations (Fig. 1, C and D) apparently all the cysts contain four nuclei (that is, two pairs), and there can be little doubt that cyst-formation is preceded by conjugation of two individuals. The protoplasmic contents are sometimes shrunk away from the cyst membrane.

Macrostoma (syn. *Tetramitus* p.p.) *mesnili* (Fig. 1, E and F). This flagellate is slightly smaller than *Lamblia*. The body is pear-shaped but not flattened, and it generally possesses a tail process; it is capable of altering its form to some extent, and in faeces which have been standing for some time the body form may have become quite irregular. Contrary to what is the case in *Lamblia*,

Fig. 1.—A and B, *Lamblia intestinalis*, active forms; C and D, cysts of same; E and F, *Macrostoma mesnili*; G, *Trichomonas hominis*. × 2000.

the protoplasm of *Macrostoma* is usually vacuolated, some of the vacuoles containing ingested food masses. At the anterior end is a large, conspicuous cytostome or oral groove, inside which one of the four flagella, rather shorter than the other three, invariably lies. This flagellum, by its vibration, produces a constant flickering inside the cytostome. The single nucleus is situated quite at the anterior end of the body, and the flagella originate close to it. Even with the precaution of first fixing the smear, these parasites are too often rounded and flattened (cf. Fig. 1, F), though the flagella can usually be made out; after drying alone, before hardening, nothing is seen but a shapeless mass of protoplasm, with a large nucleus. There cannot be the slightest doubt that the parasite found by Castellani in dysenteric stools in Ceylon, and regarded by him as an entirely new type of organism ("*Entoplasma*") was one of these flagellates, probably a large form of *Macrostoma*. Cysts which may have belonged to this form have been seen very rarely. They were ovoid, but smaller than *Lambli*a cysts, and showed a single (?) dull nuclear area, somewhat nearer one end of the protoplasm.

Trichomonas hominis (Fig. 1, G).—The body is usually pear-shaped, but may be rounded. This form can be distinguished by its jerky, irregular movements, and by the conspicuous undulating membrane running along one side. The vigorous action of this membrane often produces waves of the peripheral protoplasm, passing down the side of the body, from before backwards. The membrane ends posteriorly, and its flagellar border is continued as a short free flagellum. Three anterior free flagella are present, which can be made out in sluggish individuals. In the stained individual of Fig. 1, G, the curved wavy line on the right is the attached flagellum, which terminates freely to the left. The inner line running roughly parallel with the attached flagellum is the so-called axial line, marking the base of the membrane. The shorter curved line on the left starting from the nucleus, is the single axial rod; in life this projects backwards, forming a stiff tail to the body. Up to the present the cysts of this form have not been observed.

Cercomonas hominis.—Only on one occasion has an infection—a very slight one—with this flagellate been observed. A single active individual was found; it was very amoeboid, but the main flagellum could be seen vibrating freely, thus distinguishing the creature from an amoeba. A scanty number of cysts were present. These are spherical, with finely granular contents. They are slightly larger than the other flagellate cysts, but distinctly smaller than an *Entamoeba* cyst; there is only a single nucleus in the cyst, not always clearly visible. This form is a true human parasite, distinct from the coprozoic form, *C. longicauda*, which passes through the bowel in the encysted state and is only active in faeces (cf. the case of water Amoebae and parasitic *Entamoebae*).

Coccidian Cysts.—Human coccidiosis has always been regarded as a very rare and exceptional occurrence. In

Fig. 2.—Coccidian cysts: A, containing a single protoplasmic mass; B, with two masses (sporoblasts). Drawn from life. $\times 1000$.

the faeces of 5 cases bodies have been found which are in all probability coccidian cysts (Fig. 2, A and B). They were usually scanty, but in one case not infrequent, five or six cysts being quickly found in one fresh preparation. The cyst is ovoid, with rather narrow ends. The membrane is definite and firm, but rather thin. The size of the cyst is very generally about 32 by 14 microns. It is thus little more than half the size of any human nematode ova, according to textbooks of parasitology; on the other hand, the dimensions are well within the limits of those given for the cysts of coccidia which have been described from man. Further, the contents of the cyst appear quite different from those of eggs. The cyst contains either one or two protoplasmic masses, larger or smaller respectively, which are full of large refringent granules, strongly resembling the characteristic reserve food granules of

coccidian oöcysts. More than two masses have never been observed—that is to say, this does not appear to be the segmentation process of an egg. Nor have any worms been found in a single case. The two masses, resulting, doubtless, from the division of the single larger one, represent most probably two sporoblasts, which will become the sporocysts. For some reason or other no further development has ever been observed in cysts taken from faeces, after the latter have been kept for a day or two. The coccidian is apparently of the *Iso*spora type rather than the *Coccidium* type, which has four sporocysts; a species of *Iso*spora is known to occur frequently in cats and dogs. There has been so far no opportunity of determining whether this coccidian infection is restricted to the intestinal epithelium or whether it also invades the liver.

Blastocystis hominis.—This peculiar parasite, which has been known only a few years, is usually regarded as allied to the fungi (Ascomycetes) rather than to the protozoa. It has been undoubtedly the cause of much confusion, and has been mistaken for resting or resistant stages of different flagellates—for example, *Trichomonas* or *Macrostoma*. In one case, where the stool was very watery and of a clayey colour, enormous numbers of this parasite were present. Different stages of *Blastocystis* are seen in Figs. 3, A and B. The body is spherical, motionless, and

Fig. 3.—*Blastocystis hominis*: A, with a single nucleus; B, with two. $\times 2000$.

in the earlier stages shows a large vacuole; frequently, in fact, the body appears to be nearly all vacuole, there being only a narrow rim of protoplasm enclosing it. The vacuole is clear, and not fatty or oily in appearance. The limiting membrane of the body is thin and delicate, and does not resemble the cell wall of a fungus. The parasite is probably more closely allied to the protozoa than to the yeasts or blastomycetes. At first there is only a single nucleus, lodged in the rim of protoplasm. With increase of size the nucleus divides, the two daughter nuclei pass to opposite sides of the body, and simple binary division may take place. Or nuclear multiplication may continue, and the vacuole may diminish and tend to disappear as such. This condition probably leads to multiple fission. Resistant stages, or cysts, have not yet been observed.

Entamoeba coli.—Up to the present no cases of infection with *Entamoeba tetragena* (the cause of amoebic dysentery) have come under notice. In several cases, however, the cysts of *E. coli* have been found in the faeces, usually in scanty numbers, but once or twice fairly numerous. Active forms have not been observed at all. The cyst of *E. coli* is usually spherical (Fig. 4), but may be ovoid. It is considerably larger than any of the flagellate cysts, and is further distinguished from the latter by the fact that the membrane is thick, with a well marked double contour. By careful focussing, moreover, many nuclei, appearing as small rings slightly clearer within than the surrounding protoplasm, can be made out. Practically all the cysts in which the nuclei could be clearly made out had from six to eight nuclei, the latter being the normal complete number. This fact, of course, is usually given as a definite diagnostic character distinguishing *E. coli* cysts from those of *E. tetragena*, which have four nuclei as the complete normal number. All the evidence obtained so far supports the view that *E. coli* is non-pathogenic, and a distinct form from *E. tetragena*.

Fig. 4.—Cyst of *Entamoeba coli* with eight nuclei. Drawn from life. $\times 1000$.

Pathogenicity.

With regard to the question of the pathogenicity of the above flagellates, the data so far to hand afford only slight indications. Of late years, several Continental workers have recorded cases of "flagellate-dysentery" (the flagellates being usually *Lambli*a or *Trichomonas*),

which have been chronic, giving rise to continued diarrhoea, often with blood and mucus in the stools, and proving refractory to ordinary methods of treatment. These cases have been regarded as not due to bacillary or amoebic dysentery, but precise evidence as to the absence of the former is usually lacking. The present series of cases is complicated by the fact that, in the majority of them, one or more types of pathogenic bacteria have been obtained. Blood, mucus, and pus stools have occurred only in two cases, from both of which Shiga's bacillus was recovered. On other occasions, where loose stools have occurred, at times slightly mucoid but with no blood or pus, Shiga has either been obtained or, where not actually recovered, the patient's serum has subsequently given the agglutination reaction. Or, again, some other organism—for example, *B. paratyphosus* A—has been present. In several cases, in fact, the patients appear to have been infected with several different organisms, bacteria and protozoa. It is quite possible that the bacterial infection and the disturbance caused by it have favoured the establishment of the protozoan infection, whatever this has been.

Nevertheless, there is a residuum of cases, where loose diarrhoeal stools have occurred, containing flagellates or their cysts, where no evidence of pathogenic bacteria has been obtained. Where active flagellates are present the motions are nearly always loose or thin and creamy or bright yellow in colour, the latter being markedly the case with stools containing *Trichomonas*. This parasite has occurred in diarrhoeal stools from one patient at intervals over eighteen days, after which it apparently died out. Up to the present this form has not proved persistent or refractory. *Lamblia* is probably a more important cause of intestinal derangement, and this can be readily understood when the essential parasitic character of this form and the situation it occupies are borne in mind. The following is the record of one of three very similar cases of infection with this parasite, uncomplicated (now) by the presence of any other pathogenic organism. In the instance in question, the active forms have never been seen in the stools examined; this fact shows the importance of the recognition of the cysts.

W—s. Admitted September 26th, 1915.

September 28th. Pasty yellow stool; fair number of *Lamblia* cysts.

October 13th. Loose, slightly mucoid stool; no blood or pus; some *Lamblia* cysts.

October 22nd. Pasty, olive-green stool; very numerous *Lamblia* cysts.

October 30th. Thin, soup-like, greenish-brown stool; no blood or mucus; numerous *Lamblia* cysts.

The presence of cysts in such numbers indicates, of course, a heavy intestinal infection with the active forms. During this period the patient has suffered from chronic diarrhoea; during the night before the last sample was examined, for instance, he had five thin or loose motions. Prior to admission, the patient, who came from Gallipoli, had a history of six weeks' dysentery and diarrhoea, with blood and mucus in the stools during the first two or three weeks. During this early period he had three or four injections of emetine. Probably, therefore, the patient had amoebic dysentery, which was entirely cured by the emetine, for neither *Entamoebae* nor their cysts have ever been found in the stools. The emetine had apparently, however, no effect upon the *Lamblia*. French workers strongly recommend turpentine (térébenthine) in cases of persistent diarrhoea due to these flagellates. It is hoped to obtain further information upon this subject as time goes on.

REFERENCES.

¹Ledingham and Arkwright: *The Carrier Problem in Infectious Disease* (Arnold), 1912. ²Wien. klin. Woch., 1915, pp. 579, 620.

ACCORDING to the New York Medical Record, the field service of the American Ambulance in Paris during the first eleven months of its activity transported 57,000 wounded from the front. The number of automobiles now employed in this service is about ninety, and, in addition, twenty machines are attached to the hospital at Neuilly. One supply and one staff machine accompany each of the units in the field, themselves composed of from ten to twenty-five cars. There have been no deaths among the drivers of the field ambulance, although they go very near to the fighting lines and are frequently under heavy fire. More cars are added to the field equipment as the fund grows. The number of beds in the hospital at Neuilly has been increased from 175 to 600.

NOTE ON THE TETRAVACCINE: TYPHOID + PARATYPHOID A + PARATYPHOID B + CHOLERA.

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It may be of interest to put on record the somewhat extensive experience we have had in Serbia with the tetravaccine: typhoid + paratyphoid A + paratyphoid B + cholera, one of the combined vaccines prepared and used for several years in Ceylon by one of us.

Technique of Preparation.

This is identical with that used by one of us previously, and already published in various papers. It may, however, be of advantage to describe it again briefly.

The growth of typhoid cultures is washed off with sterile 0.85 per cent. salt solution, to which 0.5 per cent. carbolic acid has been added; the emulsion so obtained is stored at room temperature (18° to 20° C.) for twenty-four hours, and then standardized. To standardize it the germs are counted by using a Thoma-Zeiss apparatus, and sufficient carbolic salt solution is added to bring the number of germs down to 2,000 millions per cubic centimetre. The standardized emulsion is tested for sterility. The same procedure is carried out with paratyphoid A and paratyphoid B cultures, these two emulsions being also standardized to contain 1,000 million germs per cubic centimetre. The above procedure is also carried out with cholera, the emulsion of which, however, is standardized to contain 4,000 million germs per cubic centimetre. The four standardized emulsions when found sterile are mixed together in equal proportions, and the vaccine will therefore contain per cubic centimetre:

Typhoid	...	...	...	500 million
Paratyphoid A	...	...	...	250 "
Paratyphoid B	...	...	...	250 "
Cholera	...	...	...	1,000 "

Of this mixture 0.5 to 0.6 c.c.m. are given under the skin of the arm the first time, and the same amount a week later. A third dose, also $\frac{1}{2}$ c.c.m., given two weeks after the first, is of advantage, but not essential for practical purposes.

The Peptone Water Method.—Owing to the scarcity of agar at one time in our laboratory we prepared a certain amount of vaccine with peptone water in the following way. Large bottles were filled with peptone water containing 1 per cent. peptone and 0.75 per cent. sodium chloride, and sterilized. Some of the bottles were then inoculated with typhoid, others with paratyphoid A, others with paratyphoid B, and others with cholera. The cholera bottles are kept in the incubator at 35° C. for twenty-four hours. The bottles inoculated with typhoid, paratyphoid A, and paratyphoid B were incubated for forty-eight hours, as the growth of *B. typhosus* and allied germs is slow and scanty in such a medium. We found by experience that with the peptone water we used and with the strains of germs we had, provided the bottles were kept in the incubator exactly the stated period of time and temperature, the number of germs was, for practical purposes, nearly constant. The cholera bottles will contain approximately 3,800 to 4,000 million cholera vibrios per cubic centimetre and the typhoid and paratyphoid bottles will contain approximately 900 to 1,000 million per cubic centimetre. The peptone water cultures were then mixed in the following proportions:

Typhoid	...	...	...	2 parts
Paratyphoid A	...	...	...	1 part
Paratyphoid B	...	...	...	1 part
Cholera	...	...	...	2 parts

To this was added $\frac{1}{2}$ per cent. carbolic, which is sufficient to sterilize the vaccine in a very few hours. We may say at once that the results obtained with this vaccine as regards agglutinins, etc., appear to be very