

REPORT OF A BASE LABORATORY IN MESOPOTAMIA FOR  
1916, WITH SPECIAL REFERENCE TO WATER-BORNE  
DISEASES.

BY CAPTAIN T. K. BONEY.

*Royal Army Medical Corps.*

CAPTAIN L. G. CROSSMAN.

*Royal Army Medical Corps.*

AND

LIEUTENANT (U.L.) C. L. BOULENGER.

THE following is a short account of the results obtained in the laboratory at — British General Hospital, dealing with the diseases prevalent in Mesopotamia among the troops during 1916. A few brief remarks as to climate and general considerations may be of service to a preliminary understanding of the prevailing conditions there.

The months of January, February and March constitute the period of the rains. During the whole of this time the river is rising, and continues to do so until about the middle of May, after which it gradually falls, the lowest level being reached by about the middle of October. During the first three months the temperature is never very high during the day time—the average daily maximum being about 66°, 70°, and 78° F. respectively for these months. There is a considerable drop at night, though it is rare for the thermometer to fall much below 40° F. April to September constitutes the hot weather period, the average daily maximum being highest during July. In July, 1916, this daily average, taken under the prescribed conditions of shade, etc., was 110, but under the conditions in which men frequently lived, it was certainly much higher.

The first part of the hot weather is characterized by great moisture, partly owing to the large amount of flooded country produced by the swollen river overflowing its banks, and partly to the frequency with which the wind is in a southerly direction at this time. About the middle of June the prevailing wind changes to the north, causing great improvement in the climatic conditions, and continues fairly regularly from this quarter for six weeks or more. In 1916 this north wind was late in its arrival, and did not commence to blow until the end of the third week in July.

Flies begin to multiply in March, and become a great pest in April and May, then disappearing rapidly as the heat increases.

The low-lying swampy ground provides a breeding place for mosquitoes although, as pointed out by Major Christopher, I.M.S., owing partly to its being tidal, and from other causes, it is not as well adapted to this purpose as might at first sight seem to be the case.

Under these conditions it is not surprising to find that malaria and the water-borne diseases should account for such a large part of the sickness

among the troops. The latter group showed a steady increase from March onward, reaching a maximum in July and August.

The great bulk of the material sent for examination was supplied by this hospital; a certain amount, however, came from other units in the vicinity not supplied with fully equipped laboratories. This was chiefly of the nature of blood cultures and agglutination reactions.

#### ENTERIC GROUP DISEASES.

A chart showing the monthly incidence of enteric group disease, as diagnosed by blood culture in this laboratory, is shown at the end of this section. Although over 650 cases have been diagnosed by all methods during the year, only 21 per cent were found to be true typhoid, while 65 per cent were due to *Bacillus paratyphosus* A.

As far as we can determine, paratyphoid B fever had not been diagnosed in this country prior to the arrival of the — Division in March. We (K.B.) had not isolated the organism, and had failed to find agglutinative evidence of its presence in the blood; and we believe our predecessor had met with a like result. On the arrival of the — Division many cases of enteric and both paratyphoid fevers were admitted from the transports, and, apart from agglutinative evidence, *B. paratyphosus* B was isolated by blood culture on two occasions during this month from among these cases.

It would appear that an orderly nursing in the paratyphoid ward of this hospital was the first case of paratyphoid B infection arising in this country to be diagnosed as such. During April a few cases (five) continued to be diagnosed, and an increase occurred in May which reached its maximum in June. In fact, more than fifty per cent of all the cases diagnosed occurred during these two months, blood cultures being positive on several occasions. Since then the organism has never been isolated from the blood, and its incidence, judged by agglutination tests, has fallen to four or five cases a month. Nearly all of these latter came from the Euphrates area. The rise of paratyphoid A fever during the hot weather, reaching its maximum in September, is also shown on the accompanying chart.

Investigation was carried out under three headings:—

- (a) Isolation of the organism from the blood.
- (b) Isolation of the organism from the stools.
- (c) Agglutination reactions.

Urine was only examined on five occasions with a negative result in each case. Blood cultures have been made systematically on all possible cases. None were made in January or February, as no acute cases in early stages of the disease were seen then.

Stool examination was commenced in January, and carried on intermittently until the end of April. During the hot weather it practically ceased, and was resumed again at the beginning of October. It has never been done systematically in all cases, but was chiefly relied upon in those seen too late for blood culture, in which agglutination reactions were likely to be modified by reason of inoculation with T.A.B.

Agglutination reactions were performed on all cases not coming in previous groups.

*Blood Cultures: Technique.*—Five cubic centimetres of blood were withdrawn from a vein and inoculated into ten cubic centimetres pure ox bile. This latter could always be obtained from the slaughter-house, and proved a very satisfactory medium. As a general rule, blood was not taken before the fourth day, nor after the tenth. Relapses, in cases not previously diagnosed, were made an exception; and in many such a positive result was obtained late in the disease.

After incubation for twenty-four hours, and at the end of every subsequent twenty-four hours for four days, subcultures were made into mannite peptone water. As soon as evidence of acid formation was seen, an agar slope was inoculated from this tube, and also a broth tube. This was examined for motility, and the slope was emulsified at the end of twenty-four hours, and tested against high titre serum in dilutions up to 1 in 800 or 1 in 1,600. Usually a dilution of 1 in 800 with the serum was considered sufficient.

Identification was thus based on:—

- (1) Reaction and amount of gas, if any, in mannite tube.
- (2) Character of growth on agar and motility.
- (3) Reaction to specific serum.

At first other sugars were used, but the purity of some of them was not above suspicion, and for the identification of an organism isolated from the blood-stream, their use was not deemed essential as a routine measure. The high titre serum was of Lister Institute manufacture, and was the ultimate criterion of specificity. It was kept in the ice-chest during the hot weather. The sugars, dulcitol, lactose, glucose, and salicin were employed if any irregularity was observed in (1) or (2), or if complete agglutination in dilution 1 in 800 did not occur in two hours at 37° C. If excessive gas was observed in the mannite tube, or early formation of gas, plating on MacConkey was always done to avoid masking of E. group organisms by contamination.

The behaviour of the paratyphoids in mannite peptone water differed markedly. As a rule *paratyphosus* B formed acid and gas vigorously—usually to the extent of about one-third of the Durham tube in twenty-four hours.

Of ninety-five strains of *paratyphosus* A there was no gas formation at all in twenty-eight. In sixty-four, there was slight gas formation at the end of twenty-four hours, very often merely a bubble. At the end of forty-eight hours this would have increased to about one-third of an inch. Two others showed marked gas formation, and in one the gas more than half filled a Durham tube. These latter gave the ordinary fermentation reactions of the group.

A certain number of anomalous organisms were isolated from time to time, which could not be referred to any group, although from the delicate

nature of the growth and other reasons, they did not appear likely to be contaminations. Two were submitted to Parel Laboratory for animal inoculation and further report, and a good many others could not be worked out for want of time.

In four cases organisms giving all the sugars reactions of the paratyphoid group were met with, but which did not agglutinate or only did so very slightly. The figures dealing with positive results do not include any of these. On one occasion hæmoculture yielded an organism of *Fæcalis alcaligenes* group.

At the beginning of October, it was arranged to issue for blood cultures small sealed bottles of ox bile to other units on application. The result was communicated by telephone, but percentage of positive results compared with those done in this hospital has been very small. This may in part be due to bad selection of cases by medical officers, but may also be accounted for by delay in reaching the laboratory.

	B.G.H.	Other sources	Totals
Total number of cultures made	352	32	384
Positive to <i>B. typhosus</i>	21	1	22
„ <i>B. paratyphosus</i> A	93	3	96
„ <i>B. paratyphosus</i> B	7	0	7

Inagglutinable strains resembling paratyphoids in all sugar reactions, 4; and *B. paratyphosus* B more closely in respect of gas formation.

The figures show that 32.6 per cent of positive were obtained of all cases, whereas taken separately only 12 per cent of outside cases yielded positive results as against 34.4 per cent of cases from this hospital.

The relative incidence of the members of the group deduced from these figures is: T., 17.6 per cent; A, 76.8 per cent; B, 5.6 per cent. The figures for paratyphoid B are low when compared with the agglutination results. It is probable that the inagglutinable strains, had it been possible repeatedly to subculture them and test them against the serum, would have been found to agglutinate and should also be included among them.

Further, the shorter duration of the bacillæmia in paratyphoid B infections compared with *B. typhosus* and paratyphoid A infections has been reported upon, and in cases received at the end of the first week or later, for this reason alone the diagnosis of the infection by blood culture would not be on all fours with diagnoses made in the same way in the case of other members of the group.

Nevertheless the duration of the bacillæmia, speaking of the group generally, is in some cases strikingly long, and in most cases would appear to last well over a week.

Analysis of 87 cases of continuous fever, all yielding a positive blood culture of an enteric group organism, gives the following result.

Day of disease:	3	4	5	6	7	8	9	10	11	13	15	19	30
Number of positives	1	6	12	12	18	16	9	8	1	1	1	1	1
Percentage of total blood cultures	16.5	40.	28	25	36.7	37	25	29.6					

During relapses successful cultures were made on the sixteenth, twenty-first, twenty-third and fortieth days of illness, and in a few other cases in which the duration of the illness was uncertain.

In one case *B. paratyphosus* B was isolated from the blood and *B. dysenteriae* Flexner from the stools.

*Stools.*—The difficulties in the way of systematic stool plating were considerable, principally owing to the climatic conditions. Apart from the fact that media for plates all had to be prepared and sterilized in the room where microscopic work was going on, thus adding considerably to the already high temperature, large quantities of medium could not be prepared as moulds, etc., grew very rapidly. For three months, plates would not set unless placed in the ice-chest, already overcrowded, and of construction not ideal for the accommodation of a plate level in the presence of rapidly melting ice. When ready, plates had to be used the same day to give good results and the fitting in of the preparation of the plate with the arrival of the stool at the laboratory frequently left much to be desired. As a result, the method was only followed in a few special cases, reliance being placed on blood culture and Widal tests for the diagnosis of members of the enteric group.

Of 108 examinations, *B. typhosus* was recovered five times and *B. paratyphosus* A eleven times. *B. paratyphosus* B was never isolated.

*Technique.*—At first the time-honoured method of direct plating on MacConkey was used. Subsequently the modification of Browning's original brilliant green method as recently described by Leitch (*Brit. Med. Journ.*, September, 1916) was tried. The difficulty here seemed to be to hit off the right length of time in which to allow the brilliant green to act before plating. Varying periods of from ten to twenty hours were tried without much obvious advantage, and finally direct plating on MacConkey was again reverted to. It would appear in any case that the method is best suited to a search for *B. paratyphosus* B, too great inhibition being exerted on the more delicate growing *B. paratyphosus* A. At the end of twenty-four hours, suspicious colonies were fished, examined for motility, and the sugar reaction tested. The specific serum test was then applied as for organisms isolated from the blood.

*Agglutination Tests.*—The bulk of E. group cases have been diagnosed by this method. Although admittedly the least desirable means, this was only to be expected in a hospital receiving so large a number of its cases from up-river, and relatively so few direct admissions. In these late cases, recovery of the organism from the stool or urine is the only alternative, but the difficulties in the hot weather and the length of stay in hospital required, frequently renders such search impossible. Recourse therefore must be had to agglutination tests, and in T.-inoculated persons the error is probably not very great—perhaps ten or fifteen per cent. In such cases, marked agglutination to either A or B has been taken as presumptive evidence of infection by the corresponding organism, even if the T. titre was also high. The possibility of mistaking a true T. infection for A or B

under these conditions would appear remote, since it is generally agreed that T. infection produces as a rule little group agglutination for A or B, whereas in A or B infections usually the first noticeable change is a rise in the titre to be *B. typhosus*. It is much more necessary therefore to be on guard against diagnosing as a T. infection one which is really due to A or B, the true nature of the case becoming apparent later on by the appearance of agglutinins for these organisms, generally by the end of the third week.

In this way diagnoses made by us on the presence of fairly high T. agglutinins during the second week have in a certain number of cases been altered after subsequent agglutination tests have been performed. It is probably inevitable that a certain number originally so diagnosed have escaped further observation and have helped wrongly to swell the typhoid agglutination figures, although possibly not many, as a comparison of percentages with blood culture percentages will show. The short stay in hospital of many cases received from up-river prevented multiple agglutination tests being done as a routine measure. It was done however in a great number of cases, if considered necessary. In uninoculated persons and in a *certain number* of T.-inoculated persons it was thought that one Widal would suffice, if taken sufficiently late in the disease. A difficulty was met with occasionally when successive agglutination tests showed progressively increasing titre to all three organisms. In some of these in which the B titre was a good deal higher than that of A a diagnosis of paratyphoid B was made; but generally, in such, no attempt was made to diagnose specifically. Even in these cases such agglutinations are at least sufficient to indicate the presence of E. group disease.

To warrant a diagnosis of true typhoid in a T.-inoculated person, we required a high or rising titre to T. with none to A and little or none to B. With the method used, a titre of 1 in 320 was generally considered sufficient unless inoculated within eight or nine months.

The recent extensive introduction of T.A.B. has greatly impaired the value of agglutination tests in our opinion. In the majority of such cases it would appear impossible to attempt an accurate diagnosis by such means, except possibly by an elaborate series of tests, spread over a considerable period of time. At a base hospital on active service this is not practicable.

Stool examination, uncertain as a means of detecting the "carrier," is even more uncertain as a means of diagnosing large numbers of acute cases during a rush of work.

In this connexion it may be pointed out as a causal factor that whereas the suspected convalescent "carrier" can be purged with impunity, a like freedom of treatment is seldom desirable in patients during the acute stages. In the latter case one has to work with stools that may have lain in the large intestine many hours, obviously detrimental to a successful result.

Possibly more energetic hæmoculture in all suspected early cases may help in part to solve future difficulties in diagnosis.

*Agglutination Technique.*—The strains used were all reliable ones. Two were brought from France and the third was obtained from India. Carbolized agar emulsions were made from subcultures, and retained their agglutinability through the hot weather. At the beginning of September the *paratyphosus* A strain died out, and so all three were replaced by strains brought from Egypt by Lieutenant-Colonel Ledingham and which had been used there as standard. These have remained in use since with satisfactory results.

In performing the tests, the dilutions were made on a glass plate ruled with grease pencil, then drawn up into lengths of glass tubing of bore about three millimetres and incubated for eighteen hours at 37° C. when the reading was made, using a + 12 lens.

The total number of agglutination tests made during the year was 1,283, and the number of diagnoses made 520 or 40 per cent. Of these 520, enteric fever accounts for 113 = 21 per cent, paratyphoid A for 326 = 62 per cent, and paratyphoid B for 82 = 16 per cent.

*Inoculation.*—Details of inoculation have been kept on all cases investigated during the year and a glance at the results is of interest. Only those dealing with cases in which the organism was obtained from the blood will be considered.

1st Series.

		PATIENTS EITHER INOCULATED OR UNINOCULATED WITH OLD T.V.	
<i>B. typhosus</i> ..	{	Never inoculated .. .. .	21
		Never or more than eighteen months ago ..	16
		Inoculated within eighteen months ..	6
			} 22
<i>B. para. A</i> ..	{	Never inoculated .. .. .	18
		Never or more than eighteen months ago ..	30
		Within eighteen months .. .. .	49
		Not stated .. .. .	4
			} 83

In the above cases, the inoculation is either definitely stated to have been T.V. or took place at such a date that would exclude the possibility of T.A.B. Apart from the fact that cases of enteric fever only form 20 per cent of the total, it shows that of these only 27 per cent had received inoculation within one and a half years, and 50 per cent had never been inoculated at all; remarkable figures when it is considered how greatly in British troops the inoculated must outnumber the uninoculated, and therefore the correspondingly greater number of the former who are exposed to infection.

In cases of *paratyphosus* A, against which it would hardly be expected to give any protection, the incidence among the inoculated and uninoculated is seen to follow this "mass" factor just referred to, in the proportion of sixty-one to eighteen.

Figures for T.A.B. are of course only available over a short period. I give figures of all cases diagnosed by blood culture since September 1. The total number of these is fifty-three, and includes a certain number of T.-inoculated individuals already dealt with under that section.

2nd Series.		Never inoculated or not within eighteen months	.. 7	} 9
<i>B. typhosus</i>	{	Inoculated T.V. within eighteen months	.. 2	
		Inoculated T.A.B. . . . .	.. 0	
<i>B. para. A</i>	{	Never inoculated or inoculated with old T.V. . . . .	.. 31	} 44
		Inoculated T.A.B. within six months	.. 10	
		Inoculated in May and June, 1916, most probably T.A.B.	.. 3	

These figures deal with small numbers of course, but inasmuch as they are based on blood cultures they are accurate; and if they show anything they show the protective value of the old typhoid vaccine.

With regard to T.A.B., if its value be as great, it is a little surprising to find the incidence of *paratyphosus* A so high in men inoculated within six months of the onset. As a matter of fact nearly all these cases occurred within four months of inoculation, and some a good deal less than this.

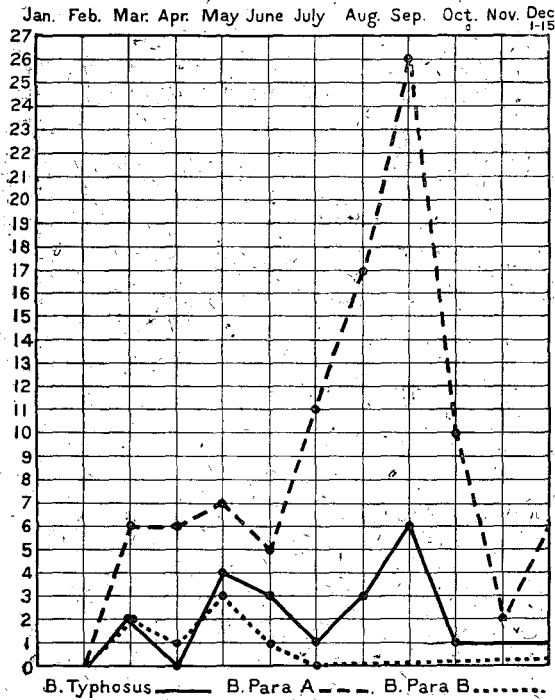


Chart showing the monthly incidence of enteric group disease by blood culture.

SUMMARY OF ENTERIC GROUP.

Total number of cases diagnosed: January 1 to December 31, 1916	.. 661	} T. .. 139 A .. 493 B .. 89
Organism isolated in 141:—		

Blood .. 125	{	T. .. 22 = 17.6 per cent total positives = 5.7 per cent total cultures.
		A .. 96 = 76.8 " " " = 25 " " " "
		B .. 7 = 5.6 " " " = 1.7 " " " "
Stools .. 16	{	T. .. 5
		A .. 11
		B .. 0

Diagnosed by agglutination:—

520	{	T. .. 112 = 21 per cent total positives = 8.9 per cent total agglutination.
		A .. 326 = 62 " " " = 25.4 " " " "
		B .. 82 = 15.4 " " " = 6.3 " " " "



#### CHOLERA.

Cholera was first noticed among the troops towards the end of April. Four or five suspected cases arrived at this hospital from up-river on April 30. A hasty microscopic examination of the stools, all of typical appearance, showed the presence of vibrios having the morphological character of cholera; a provisional diagnosis was made and the cases were transferred to a section of the isolation hospital prepared for them. From this time on, there was a steady stream of cases during the hot weather. The outbreak never reached alarming proportions and gradually died out by the end of September.

Incidentally, a mortality of about forty per cent was recorded.

A large number of stools were examined in May and June (about 120) for *V. cholerae*, many of which were positive, but unfortunately the records have been lost. Since then seventy-one stools were examined with positive results in twenty-eight. In forming a positive diagnosis, the following points were taken into consideration:—

(1) Naked-eye appearance of the stools.  
(2) Characters of smear stained with dilute carbolfuchsin, i.e., presence of vibrio forms, presence of spirochaetes (*S. euglyrata*), etc. Although the circumstances of the occurrence of the latter in the stools are but imperfectly understood, in actual practice it was found that in smears showing them a vibrio could generally be isolated in peptone water from the stools. Stools containing them were therefore regarded with grave suspicion, and a report given accordingly before waiting for the result of culture.

(3) Culture in peptone water and examination of the top layer for vibrios after eight to ten hours' incubation. The use of Witte peptone, of which we always had a supply, was found invaluable in this connexion, and frequently gave positive results when other good peptone, although sugar-free, was negative. The formation of a surface pellicle did not seem to be very constant.

(4) Reaction against specific high-titre serum. This was always done microscopically by hanging drop. Immediate complete agglutination in dilution of 1 in 200 or 1 in 400 was taken as positive.

(5) Cholera-red reaction. This was done on every case at first, but was later given up owing to its uncertainty in cases which otherwise conformed to type. The hæmolytic test was not done in any case.

Of the twenty-eight cases in which a vibrio was isolated, twenty-two were diagnosed on the growth of a vibrio in peptone water, which was morphologically *V. cholerae*, and which was agglutinated in the manner specified. In the remaining six no agglutination occurred.

#### THE DYSENTERIES.

Whether or no amœbic dysentery is endemic in this country we do not know, yet certainly it has been responsible for a great deal of sickness among the troops here throughout the hot weather. Of even commoner

occurrence has been the bacillary form of the disease according to our own figures. Speaking generally of cases with dysenteric symptoms, the maximum of the curve of incidence was reached in August and September, as in the case of enteric group disease, after which it gradually fell again.

It is to be regretted that these cases were not examined systematically from the beginning, but pressure of work and the trying conditions of heat prevented this. A small number of stools were plated (fifty-nine), all of them containing blood and mucus. *B. dysenteriae* Flexner was isolated in seven, and *B. dysenteriae* Shiga in two. Microscopical examination of eighty stools also containing blood and mucus showed *E. histolytica* in eleven.

By the addition to the laboratory staff of Captain Crossman, R.A.M.C., and Lieutenant Boulenger, a detailed examination of all cases became possible. This was undertaken at the instigation of the Medical Advisory Committee, and was commenced in September. The protozoological findings were the work of Lieutenant Boulenger, and a short account of the work is appended by him.

In this way the stools of 890 cases of intestinal disorder were investigated during the last four months of the year.

These cases fall into two groups:—

(1) Cases of acute dysentery.

(2) Cases in which some diarrhoeal symptoms were present, and in which there may or may not have been a history of a previous acute dysentery.

This latter group doubtless included a great many cases of ordinary simple diarrhoea.

The method adopted was briefly as follows:—

(1) Microscopical examination was first made on every case, and the protozoological findings noted.

Those containing blood and mucus (i.e., group I, acute dysentery), and not showing the presence of *E. histolytica*, were then plated.

(3) Stools containing no blood or mucus were not plated.

This was the general procedure, from which there were a few exceptions made.

(a) A small number (twelve) containing blood and mucus, in which *E. histolytica* had already been found, were also plated.

(b) Nineteen stools containing no blood or mucus were plated.

The results obtained will be considered according to this grouping:—

*Group I:* Acute dysentery, i.e., cases passing blood and mucus in stools. The number of such cases was 309.

*Microscopic Examination.*—*E. histolytica* 80 = 26 per cent. These are considered in the section dealing with the protozoological findings. Of the remainder 209 were plated with the following result:—

<i>B. dysenteriae</i> ..	{	Shiga .. .. .	65	} = 108
		Flexner .. .. .	38	
		Shiga and Flexner .. .. .	5	
		Negative .. .. .	101	

Thus 108 cases, or 51·7 per cent of those submitted to bacteriological examination, were proved to be due to infection by *B. dysenteriae*; and the relative incidence of the two forms of the disease will be: amœbic 26 per cent, bacillary 35 per cent, of the total number of cases examined.

Two cases of mixed amœbic and bacillary infection were noted, viz., (1) *E. histolytica* and *B. Shiga*; and (2) *E. histolytica* and *B. Flexner*.

There still remains 39 per cent of acute cases in which the causal agent was not determined. A bacillus of the Morgan No. 1 group was isolated once, but it is possible that if repeated examinations had been made a certain further proportion of these undiagnosed cases would have been cleared up. "Repeat" examinations for protozoa on negative cases were made a great number of times, but it was of very rare occurrence that *E. histolytica* was noted in any of these subsequent examinations. On the other hand, only twelve cases were plated a second time, the first occasion being negative, and in three of them a bacillus was isolated (*Shiga*). We are, therefore, inclined to think that the undiagnosed cases were chiefly of bacillary nature, either due to one of the recognized strains which a subsequent plating might have revealed, or else caused by other bacillary agents of hitherto unrecognized specificity.

From an examination of the above figures, the Medical Advisory Committee reported as follows:—

"It is thus apparent that bacillary dysentery is the predominant type in the Mesopotamian area (as in other war areas), and there is little doubt that, had the cases investigated been in the main local admissions instead of transfers from up-river, the proportion of bacteriologically proved bacillary cases would have been still higher."

*Technique*.—A small portion of mucus was emulsified in sterile saline, and a couple of large loopfuls transferred to a MacConkey plate, a piece of capillary tubing bent at right-angles being used as a spreader. After eighteen to twenty hours' incubation suspicious colonies were picked off, about three from each plate—and each one inoculated into mannite peptone water and broth. The broth tube was examined for motility, and an agar slope was inoculated from the broth or mannite tube, if no gas formation had taken place in the latter. The following day the agar slope was emulsified and tested against high-titre serum.

The inoculation of a broth tube was frequently omitted later on, as it was found that examination of the mannite tubes for motility could be relied upon, if not done later than eight or nine hours after inoculation. In the case of the non-acid forming *Shiga* group, the examination could be made as from a broth tube.

The reaction against specific high-titre serum was done macroscopically in every case, using the technique adopted in all other agglutination tests, and already described. The titre of the serum was rarely higher than 1 in 1,000 and was frequently not more than 600 or 800; and it was always found to give more marked agglutination against organisms of the *Shiga* group than against those of the *Flexner* group.

Complete agglutination in a dilution of 1 in 200 at 37° C. was accepted as positive in the case of an organism which otherwise satisfied the requirements. In the majority of cases tried, the organisms have agglutinated up to the full titre of the serum, especially those of the Shiga group.

The sugar reactions at first, after the primary differentiation by mannite, were not fully investigated. A great many later strains, however, have been kept and the sugar reactions tested against glucose, maltose, saccharose, inulin, dextrine and lactose, and subsequently against dulcite, salicin and adonite. Organisms of the Flexner group all produced acid in mannite, maltose and glucose, usually in twelve hours, and remaining acid up to five to seven days, after which observation ceased. In dextrine, acid formation was usually slight though definitely present. Most of the strains tested produced acid in saccharose within twenty-four hours. One subsequently became neutral, and in two more no change took place at all; in lactose there was no change, nor in salicin, inulin, adonite and dulcite.

Of the Shiga group, all produced acid in glucose, in maltose, acid at first becoming neutral after four or five days, or less definitely acid; in saccharose, usually no change, but acid formation took place in two at the end of twelve hours; no change in mannite, lactose, dulcite, salicin, inulin, dextrine and adonite.

A certain number of strains were obtained in which agglutination was incomplete, very slight or absent. Some of them were put through other sugars, glucose, maltose, saccharose, and dextrine, but whether they conformed to type in these reactions or not, they were not regarded as true strains. In such cases, a diagnosis of "bacillary dysentery" (mannite-fermenting group), or the reverse, was returned. None such are included in the above figures. The medium used for plates was MacConkey; Conradi-Drigalski was used earlier in the year, but was given up in favour of the former.

*Group II:* Chronic dysentery and diarrhoeal conditions, i.e., stools containing no blood or mucus.

It has already been mentioned that nineteen stools containing no blood or mucus were plated.

They were all negative.

#### PROTOZOOLOGICAL FINDINGS IN GROUPS I AND II.

From the beginning of September, 1916, onwards the stools of all patients suffering from dysentery and allied intestinal disorders were examined for pathogenic and other protozoa; this section furnishes a preliminary account of the findings from that month to the end of the year.

During this period nearly 1,300 stools were dealt with, representing 890 separate cases—all British patients.

With few exceptions the cases were patients resident in the hospital; a uniform method of collecting specimens was therefore found desirable and was briefly as follows:—

Bed-pans containing the fæces were taken by the ward orderlies to a small shed conveniently situated and set apart for the purpose. The contents were inspected at intervals during the day and the macroscopical characters—presence or absence of blood or mucus, colour, consistence, etc., noted. Small samples were taken to the laboratory in corked tubes and examined microscopically before being passed on to the bacteriologists. By this method perfectly fresh specimens were dealt with, this explaining the rather high percentage of free living organisms, e.g., free amœbæ and flagellates, observed.

Smears from the fæces were examined in salt solution (warmed to blood temperature when necessary during the winter months) and Weigert's iodine solution, according to the method recommended by Lieutenant-Colonel C. M. Wenyon, R.A.M.C., in his recent publications on the subject.

*Entamœba histolytica*.—Of the 890 cases examined 142, or approximately 15·9 per cent, were found to be passing the pathogenic *E. histolytica*, either in the free living or encysted condition.

The 890 cases naturally included a large number of convalescent dysentery patients as well as a few cases of simple colitis and diarrhœa; the acute cases of dysentery (i.e., in whose stools pus cells and red blood cells were found) numbered 309 and *E. histolytica* was found in 80 of these.

It is therefore possible to state that over a quarter (25·9 per cent) of the acute dysenteries at this hospital during the last four months of the year were of amœbic origin.

*Non-pathogenic Amœbæ*.—The non-pathogenic *E. coli* was met with in 162 cases, or about eighteen per cent of the total number. The only other parasitic amœbæ noted were small forms of the *limax* type which occurred in four cases; these are probably to be referred to the species *E. nana* recently discovered by Lieutenant-Colonel Wenyon in Egypt.

*Flagellates*.—*Lamblia (Giardia) intestinalis* was found to occur frequently and was recorded in eighty-eight, or nearly ten per cent of the cases; the pathogenicity of this species has been much discussed; there seems, however, little doubt that certain persistent diarrhœas were due to its presence in the intestines.

*Trichomonas intestinalis* and *Tetramitus (Macrostoma) mesnili* were also occasionally met with, occurring in 8·1 and 5·7 per cent of the cases, respectively.

*Coccidium*.—A *coccidium* of the *isospora* type was found in seven cases; no special symptoms of coccidiosis were, however, noted in any of these.

TABLE SHOWING THE OCCURRENCE OF PARASITIC PROTOZOA IN 890 CASES OF INTESTINAL DISORDER.

	Number	Percentage
<i>Entamœba histolytica</i> .. .. .	142	15·9
" <i>coli</i> .. .. .	162	18·2
" <i>nana</i> .. .. .	4	0·45
<i>Lamblia intestinalis</i> .. .. .	88	9·9
<i>Trichomonas intestinalis</i> .. .. .	72	8·1
<i>Tetramitus mesnili</i> .. .. .	52	5·7
<i>Coccidium (Isospora sp.)</i> .. .. .	7	0·8

## MALARIA.

In January benign tertian parasites were found in only 11 cases and subtertian in 1, out of a total of 90 examinations. An increase was not noticeable until May when the number of examinations had risen to about 150 with about sixteen per cent positive. In July, 50 positives were recorded among a total of 248 and of these 44 were benign tertian and 4 subtertian. The remaining two appeared to be the quartan variety, a marked chromatine band being present.

Subsequently to this, the level remained fairly constant with a slight drop in August. In November the subtertian had increased and were in the proportion of one to three of all cases diagnosed.

For the months January, February and July to December 15, i.e., seven and a half months, the total figures are 1,477 examinations with 260 positive = 18 per cent. Of these 260, 40 = 18 per cent were subtertian. Actual figures for the remaining months, March, April, May and June are not available. The monthly average was 200 per month with seventeen per cent positive. The total number of slides thus examined was between 2,200 and 2,300 during the year.

## RELAPSING FEVER.

Forty-seven cases were diagnosed by finding spirilla in the blood. All of these cases except two arrived with the — Division from Egypt and were men of the — Regiment who had all travelled together in the same ship to Mesopotamia. The two cases excepted were officers who had been doing duty at a Turkish prisoner's camp, where a good number of cases had occurred among the prisoners. Only one case terminated fatally. Strict isolation and disinfection of kit succeeded in limiting the epidemic to this one regiment, and in eradicating it in less than a month. Apart from this outbreak, *S. recurrentis* was not reported in this laboratory during 1916.

## JAUNDICE.

Blood cultures were done on five cases of jaundice with low pyrexia. All were negative.

## CEREBROSPINAL FEVER.

The meningococcus has been found in the cerebrospinal fluid of six cases, one of which occurred outside the hospital (Civil Surgeon's case). It could not be cultivated on blood-smear agar on any occasion, although once or twice it was present in enormous numbers.

In every case the diagnosis was based upon the polymorphonuclear nature of the exudate, and the presence of a Gram-negative diplococcus morphologically resembling the meningococcus, a certain number of which were inside the cells.

During October, the throats of fifty-one healthy N.C.O.s and men of the Australian Wireless Section were examined for the presence of the meningococcus, with negative results.

#### DIPHTHERIA.

This disease has not been of unfrequent occurrence. Altogether twenty-two cases have been diagnosed, but the majority of these have been in men of the same regiment and occurred during August and September. During the hot weather, when serum media were difficult to prepare, and still more difficult to keep, many successful inoculations were made on the white of a recently hard-boiled egg inverted in a urine specimen glass containing a little water to keep it moist. While not growing quite so readily as on serum, this method proved of considerable practical value.

#### ORIENTAL SORE.

Although smears from scrapings of suspected sores have been examined from time to time, no Leishman bodies have been seen in any of them.

#### PLAGUE.

Only on one occasion has material been sent to the laboratory from a case of suspected plague and the report was negative.

A certain number of rats have been examined however, thirty-eight in all, since the middle of October. Only one of these was caught in a trap. The others were either killed by sticks, or shot, with the exception of two which were found dead. (A great many others, not included in the above, were found dead, but were too mummified or decomposed to permit of satisfactory examination.) Six of the thirty-eight showed plague lesions, and were seen by the officer on special plague duty, Major Kunhardt, I.M.S. *B. pestis* was isolated from the heart blood in all of them.

#### VACCINES.

Prophylactic vaccines were made for cholera and enteric group. The preparation of a cholera vaccine was undertaken in May. It was an unheated agar emulsion killed by one per cent phenol and in which four strains were used in the manufacture, three of which were local, and the fourth Indian. Fifteen thousand doses were issued, the bacillary content being 2,000 million per cubic centimetre.

In concluding this report we are desirous of expressing our thanks to Lieutenant-Colonel Woodside, R.A.M.C., our Commanding Officer, for the facilities which he has always placed at our disposal for carrying on the work. To the Medical Advisory Committee, and in particular to Lieutenant-Colonel Ledingham, R.A.M.C., and Lieutenant-Colonel Wenyon, R.A.M.C., we should like to acknowledge our gratitude for their very valuable advice and help during their stay in the country; to Lieutenant Baillie, R.A.M.C., whose work in the laboratory during the most trying part of the hot weather was much appreciated, and to Assistant-Surgeon Ireland, I.S.M.D., and Corporal Muggleton, R.A.M.C., the latter for his work in connexion with plating and the collection of material in dysentery cases, the great part of which was done by him.