

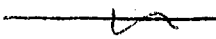
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[A STUDY OF

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[With special reference to the Cytology of Bacillary
Dysentery and its bearing on early and accurate Diagnosis.



[By

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A STUDY OF

DYSENTERY IN THE FIELD.

with special reference to the Cytology of Bacillary
Dysentery and its bearing on early and accurate Diagnosis.

Begin on p. 21

1/2 p. 9

table
2 charts
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Introductory: The campaigns of the past few years in the Eastern Mediterranean will probably stand out in medical history as a period of unusual progress in the scientific study of the clinical conditions known as "dysentery."

The troops which were landed on the Gallipoli Peninsula in the spring and summer of 1915 were collected from regions differing so widely in their climates as Scotland, Egypt, India, and Australia. Included in their numbers, there were bound to be carriers of tropical disease, and mingling as they did with thousands of unprotected individuals from the Home country, it was only to be expected that great pathological questions would arise. By the middle of July the outstanding problem with which the Royal Army Medical Corps was confronted, was the incidence, morbidity, and mortality from dysentery and seldom has the Medical profession, as a body, met a problem with more vigour and acumen.

The majority of the medical men in the East had had little previous clinical experience of sub-tropical diseases and were compelled, at first to depend upon text-books for a

description of the aetiology, clinical features, and treatment of dysentery, but unfortunately the published descriptions were somewhat unconvincing. It is only comparatively recently that anything like a scientific basis has been evolved for the proper classification of the various pathological conditions included under the heading of "dysentery"; so much so that when the results of the research conducted during the Great War came to be collected and correlated, the chapter on this disease will have to be largely re-written.

Introductory Having been attached to a casualty clearing station which landed on Suvla Bay at the beginning of August 1915, and remained there till the evacuation in December of the same year, I had an opportunity of studying the ^{dysentery} disease from the clinical point of view during its period of maximum prevalence, at a time when the morbidity rate from ^{this disease} dysentery had a very critical bearing on the military situation. The clearing station was afterwards moved to Egypt, and during the three succeeding years, had to deal with troops which included always a residue of the Gallipoli force together with reinforcements which were continually arriving from temperate as well as tropical climates. In this way I was enabled to collect material and make notes during three subsequent dysentery epidemics, namely ^{those of} ~~in~~ the Autumn of 1916 on the Suez Canal, ^{of} ~~during~~ 1917 ⁱⁿ ~~on~~ the Sinai Desert,

and during 1918 in various parts of Palestine.

A study of these notes has led to certain conclusions which have some bearing on the present-day knowledge of the disease, particularly with regard to the important field of cyto-diagnosis. The object of this paper is to describe the methods and observations upon which such conclusions are based.

Scope of the Work. This paper therefore embodies the results of work during my four years' experience of dysentery in the field. Observations during the first two years were necessarily confined to clinical and statistical phenomena. In 1917 a laboratory was attached to the casualty clearing station and from that time pathological records were always available. Special attention has been given to the subject of early diagnosis based upon the cytological examination of the cellular exudate.

SECTION II - STATISTICS.

Cf Small table.

[The importance of the disease under discussion, in its relation to military operations, is shown by the ~~following~~ figures (Table I) collected from the returns of the Casualty Clearing Station on Suvla Bay in 1915.

6p (A) (B) (C) (D), F
 - 4 -
 TABLE I

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A) Periods of four days	B) Wounds-Bullet Shrapnel Accidental	C) Intestinal diseases "Dysentery" Enteritis etc.	D) Other Diseases	Total
[Aug. 14-17, inclusive.	181	101	98	380
Aug. 18-21 inclusive.	649	160	68	877
Aug. 22-25.	514	236	179	929
Aug. 26-29.	271	384	240	895
Aug. 30- Sep. 2.	227	436	239	902
[Sep. 3-6.	111	321	230	662
Sep. 7-10.	96	193	149	438
Sep. 11-14.	66	169	170	405
Sep. 15-18.	61	202	198	461
Sep. 19-22.	54	233	179	466
Sep. 23-26.	37	201	151	389
Sep. 27-30.	98	242	195	535
[Oct. 1-4.	37	143	144	324
Oct. 5-8.	35	95	95	225
Oct. 9-12.	29	108	112	249
Totals for 60 days.	2466	3224	2447	8137
Percentages	30	40	30	100

SP-

[While the Imperial Forces were being concentrated in Egypt, that is, during the comparatively quiescent period from January to December 1916, I was afforded an opportunity for making a more comprehensive epidemiological survey of the disease and the accompanying chart (chart **1**), though limited in scope, may be taken as typical. By this time pathological laboratories had been established in the most important centres such as Cairo, Alexandria, and Port Said, and the figures quoted are all supported by bacteriological data.]

Chart I here

~~Chart I~~

[The first quarter of 1917 was a period of rapid military progress, and the climatic conditions under which the main body of the army lived, were almost ideal from a hygienic point of view. Food was excellent; flies were seldom seen; there was no call for exhaustive work on the score of military necessity; intestinal disease had practically disappeared. At the end of ^{the} Spring a very strenuous campaign was initiated in front of Gaza. The weather became uncomfortably hot; the forces were physically exhausted; the water supply was a matter of difficulty and flies began to abound. During the period of active operations, the casualty clearing Station was flooded with surgical cases, but towards the end of April the admissions were practically all medical cases,

of which intestinal conditions accounted for the great majority. A field laboratory was by this time attached to the clearing station and during the period 25th April to 8th May, 479 diarrhoeic cases were examined bacteriologically with the following resultant diagnoses:-

Cpt	Amoebic Dysentery	8
	Bacillary Dysentery (Bacilli proven)	55
	Lambliasis	1
	Acute Diarrhoea	415
	Total	<hr style="width: 100px; margin: 0 auto;"/> <u>479</u>

8/4-5 The bacillary findings again were subdivided as follows:-

No. in which organisms	isolated were	B. Shiga.	31
Cpt " " " " " " " " " "		" B. Flexner	6
		" not specified	18
		fled	
		Total	55

8/4-5 Taking only the true dysenteries found in this series, namely, Amoebic 8, Bacillary 55, the relative proportion would be -

Amoebic	dysentery,	12.7% } 5
Bacillary	dysentery,	87.3% } 5

a result which approximates the percentage recorded as the

average for all the laboratories in the previous year.

Creditable as these results are, considering the difficulties under which the work was performed, they do not fully represent the true position. Dysentery bacilli were isolated from only a small proportion of the specimens submitted for examination, and amongst the 415 cases diagnosed as "Acute Diarrhoea" there must have been a large proportion of bacillary infections.

[From the middle of May till the middle of August 1917, the troops had a period of comparative rest, and as the great heat militated against the spread of flies, there was very little sickness of ^{an} intestinal nature. The usual autumnal rise in dysentery commenced about the end of August and reached its maximum in ~~Mid~~²-October (see Chart ~~1~~²).

During this period, No. 3. Military Laboratory (o/c. Capt. P. Manson-Bahr, R.A.M.C.) was attached to the Casualty Clearing Station, and I had the privilege of combining bacteriological investigation with the clinical work in the hospital marquees.

A careful study of this Autumnal rise in the dysentery rate afforded some significant statistics, and these have been summarised in the ~~sub-joined~~ Table ~~II, F~~^{II, F}, covering the period of highest incidence, that is during the six weeks ending 3rd Nov.

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Chart 2

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From date 1st to 31st of August 1917, 28 Sept 1917, 1917

TABLE

II -

Week	Date	Total No. of Abnormal Stools Examined.	Simple Diarrhoea	Flagellate Diarrhoea	Amebic Dysentery	Bacillary Dysentery Prov. Diag.	Cultured	B. Shiga Isolated	B. Flexner-Y Isolated	
1st	23 Sept. to 29 "	57	13	2	-	42	24	10	3	
2nd	30 Sept. to 6 Oct.	86	9	4	1	72	39	9	9	
3rd	7 Oct. to 13 "	117	26	4	-	87	42	16	16	
4th	14 Oct. to 20 "	111	21	4	2	84	43	11	12	1
5th	21 Oct. to 27 "	102	24	2	2	74	38	7	16	
6th	28 Oct. to 3 NOV.	28	2	-	-	26	8	3	4	
TOTALS.		501	95	16	5	385	194	56	60	

[A striking feature in ~~the above~~ ^{T A} table is the marked preponderance of bacillary as compared with the Amoebic variety.* Taking the bacillary cases at the very minimum, that is, those in which Shiga's bacillus or the Flexner Y bacillus were isolated and gave the correct biological and agglutination tests, the numbers are:-

W

Bacillary Dysentery	- - -	116, or 95.9 %
Amoebic Dysentery	- - -	5 or 4.1 %

8 If we take the bacillary figures at their maximum, that is, including all diagnosed from a microscopic examination of the cellular exudate provisionally as bacillary dysentery, then the numbers are:-

W

Bacillary Dysentery	- - -	385 or 98.7 %
Amoebic Dysentery	- - -	5 or 1.3 %

8 Either of these findings can be taken as a significant indication of the relative importance of the two main types of dysentery in the field.

[In 1918, the troops had advanced well into the hills of Palestine, where the water difficulties of the previous year were practically at an end, where the climatic variations were less suited to the rapid increase of flies, and where the conditions of life generally were less foreign to the men from the Dominions or the United Kingdom. The result was, that from a military point of view, dysentery became a disease of secondary importance and at no period of the year did it acquire the significance of an epidemic. For the greater part of the year, I remained attached to No. 3 Military

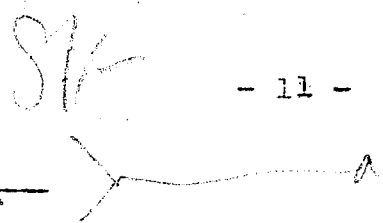
Laboratory which had moved up with the advancing troops, and the total examinations of faecal specimens for the twelve months did not exceed 2000.

In the late summer and autumn a widespread malarial infection threw a heavy strain upon all the laboratories, and little time could be spared for the culture of faeces, so that dysentery statistics are not as complete and telling as they were in the previous years of the war. Out of 2000 examinations, the cases diagnosed as true "dysentery" numbered 708. Of these only 80 or 11.3 % were diagnosed as Amoebic and 628 or 88.7% as "probably Bacillary". A certain number of the latter were cultured and in a small proportion B. Shiga and B. Flexner Y were definitely isolated.

~~Notes~~
Notes:-

7/11 Under the heading of B. Flexner Y, I have included the classical mannite-fermenting bacilli. The slight differences between the original Flexner⁽¹⁾ strain and the Y bacillus of Hiss⁽²⁾ and Russell are so evanescent that for all practical purposes they can be disregarded. Both are agglutinated in moderate dilution by the specific sera issued by the Lister Institute.

Case 7
Small Cells



Section II Cytology.

[The special investigation regarding the cytology of the stools of bacillary dysentery with a view to establishing a differential diagnosis as near the front line as possible, was carried out chiefly during the period of highest incidence in 1917. At that time the Casualty Clearing Station with the adjacent laboratory was situated about ten miles behind the active troops, and patients were usually under observation within from one to three days after the commencement of their illness. The rule was that every patient admitted with diarrhoeic symptoms was allotted a clean bed-pan, and his first stool after admission was immediately carried down to the laboratory, where the microscopical examination of the fresh specimen was carried out often within fifteen minutes of defaecation. A likely piece of the stool was selected and transferred by means of a platinum loop from the bedpan to a clean slide. A cover glass was then superimposed and gently pressed down so as to give an even transparent film, of a thickness sufficient to allow the free movement of any contained entamoebae.

Classification of Cells.

[The microscopic picture in an early case of dysentery is always a striking one, on account of the typical cellular exudate presented, and it is perhaps convenient to describe here the differential features of the characteristic cells and organisms found therein.

(1) Cells derived from the gut wall:

(a) Squamous epithelial cells.— These are derived from the margin of the Anus, are usually of horse-shoe shape, and have the characteristic large oval nuclei.

(b) Columnar epithelial cells. These are derived from the inner coating of the intestinal wall, are usually isolated, but are sometimes found in orderly clumps. Owing to osmotic pressure in the liquid stool they are often pear-shaped and the rounded nuclei are usually easily identified.

(c) Endothelial cells may be recognised in at least three distinct varieties.

(i) Plasma cells. These are larger than mononuclear leucocytes and are oval in shape. The protoplasm is not refractile and there is a large oval nucleus usually ex-centrally placed.

(ii) Macrophages^{cells}. These are derived probably from the endothelial lining of capillary walls, vascular as well as lymphatic. They are polygonal when in clumps but usually are isolated and of rounded shape. In size they are generally larger than any other cells in the stool, measuring 15-30 μ . in diameter, or even more, and have a large well-defined nucleus of reticular structure and ex-centric in position. The cytoplasm is not refractile but is often vacuolated and may contain red blood corpuscles, leucocytes and lymphocytes, that is, they are phagocytic. Their exact appearance is dependent to some extent upon the degree of osmosis which has taken place. The size and shape of these cells and the presence of included particles sometimes render it a difficult matter to differentiate them from Entamoebae, but the motility and refractility of the latter are usually conclusive, and in the living state there should be no great difficulty.

(iii) Irritation or tissue mast cells.— These are irregular in shape and about the size of a leucocyte. The nucleus is ill-defined and the protoplasm is coarsely granular. It is difficult to differentiate these cells from eosinophiles until they are stained.

II.2. Cells derived from the blood.

There are usually large numbers of red blood corpuscles in all varieties of acute dysenteric stools. The white cells are classified according to the nomenclature used in most text-books:—

- (a) Polymorphonuclear leucocytes;
- (b) Large Mononuclear leucocytes;
- (c) Small Lymphocytes; and
- (d) Eosinophile leucocytes.

3. III. Pathogenic Entamoebae.

The Entamoeba histolytica is now accepted as the sole causal agent in Amoebic dysentery, and while different phases in its life history, such as encysted or Tetragena forms and small or Entamoeba minuta forms are occasionally met with in stools that appear normal, the vegetative or active Entamoeba is present in all acute cases of ^{the} Amoebic type.

This organism is usually a noticeable object in the field on account of its refractile ectoplasm, its pale bluish-green colour, and its active streaming movements. The margin of clear ectoplasm is sharply defined from the endoplasm which is finely reticular in structure and may be vacuolated; it contains a somewhat indistinct nucleus. The endoplasm may contain ingested leucocytes, red blood corpuscles, or other body cells all more or less shrunken and disintegrated.

When the nucleus can be identified it appears to be ring-shaped with crescentic thickenings of chromatin round the nuclear membrane and its position is usually ex-centric.

IV. Other protozoal organisms such as *Entamoeba coli*, *Tetramitus mesnili*, *Trichomonas hominis*, *Lambliia intestinalis*, etc. are frequently seen, but as none of these have ever been proved pathogenic of true dysentery, that is, of an acute inflammation of the bowel, their presence may be disregarded in an investigation of this nature.

The Cellular Exudate in Bacillary Dysentery.

The exact degree of reliance which may be placed upon a diagnosis of bacillary dysentery made from a preliminary examination of the cellular exudate has for some years been a controversial point but the experience of the epidemics during the war has, I think, definitely proved its value. When a film of the blood-stained muco-pus from the scanty gelatinous stool is placed under the microscope, the most striking feature is the abundance of cells, and the scarcity or absence of digestive debris. The cells found, consist of varying proportions of the following types:-

- (a) Polymorphonuclear leucocytes. These form the majority of the cells in all stages of the disease. They may have the normal fresh appearance of healthy leucocytes, but more frequently they are swollen and altered in a characteristic manner. The cytoplasm undergoes a fatty

degeneration, the nuclei becomes distended and ghost-like, and the cell dies en masse. This appearance is very different from that found in Amoebic dysentery where the leucocytes seem to undergo a digestive process, and become progressively reduced in size owing to a marginal disintegration.

(b) Other blood cells. The red blood corpuscles vary considerably in number according to the stage and the degree of acuteness of the attack. In sub-acute or chronic cases where the stool is composed largely of mucus, there may be an entire absence of red cells in the field, whereas in Amoebic dysentery they are present in nearly all cases.

Lymphocytes and large mononuclear leucocytes are present in insignificant proportions while eosinophiles are very seldom seen.

(c) Macrophages. ^{endothelial cells} These cells form a small proportion of the total exudate, but their size and characteristic appearance mark them out for special attention. Like the polymorphs, they are found in various stages of degeneration, the cytoplasm becoming granular, vacuolated, and then hyaline; the nuclei becoming granular, distended, and finally broken up and scattered as irregular fragments throughout the cytoplasm.

- (d) Epithelial Cells, either columnar or squamous. In the early inflammatory stage of the disease, there may be very few of these cells, but in the later desquamative phase they are found often in large numbers.
- (e) Plasma Cells and Irritation Cells are Present in small variable proportions depending apparently on the depth to which any ulceration of the gut wall has taken place.

Cellular exudate in Amoebic Dysentery.

8/11 - [The differential diagnosis of the two main types of dysentery would seem to present little difficulty if one is dealing with ordinary uncomplicated cases; the microscopic examination in a fresh Amoebic specimen presents a very different picture from ^{the one} that just described. The most striking feature here is the scantiness of the cellular exudate unless, as is sometimes found, the specimen consists of almost pure blood from ulceration through the wall of a vessel. With regard to the cells that are present, those consist of:-

- 7/11 (a) Polymorph leucocytes. These are rarely seen in their normal condition. A proteolytic process is obviously at work, and they are usually partially ingested, giving them a "mouse-eaten" appearance. The process commences at the periphery of the cytoplasm and progresses until only remnants of the nuclei remain scattered throughout the film - a very different picture from that in the bacillary type where the leucocytes die by a massive toxic necrosis.

(b) Other blood cells. These also exhibit differences that are equally striking.

The lymphocytes are relatively increased in number and have the same degenerated appearance, while the Eosinophiles are markedly increased. The prevalence of Eosinophiles is so noticeable that it might almost be regarded as a pathognomic sign of the presence of Amoebae.

The red blood corpuscles also show a remarkable phenomenon. In bacillary dysentery the red cells are isolated as in diluted normal blood, but in the Amoebic type they are generally clumped together in small groups of from two to eight, suggesting some agglutinative effect. This phenomenon, so far, does not appear to have been mentioned by other observers.

(c) Macrophages ^{cells} ~~They are~~ Very rare in uncomplicated cases of Amoebic origin.

(d) Epithelial cells. ~~They are~~ Usually present together with plasma cells but there is nothing to distinguish them from those found in the desquamation of bacillary origin.

(e) The vegetative Entamoeba histolytica or its encysted form. This clinches the diagnosis of Amoebic dysentery, but where that organism cannot be found - and Wenyon⁽³⁾ has pointed out that the chances of not finding it are considerable - then in a case presenting a pathological

picture such as has just been described, one is justified in reporting it as "probably amoebic dysentery".

8/22 The recognition of the outstanding features of the cellular exudate, and their great importance as a factor in the rapid diagnosis and early treatment of two diseases differing so widely as regards their pathology and therapeutic requirements, naturally calls for a closer investigation of this field. With that object in view I arranged to make careful observations in a series of suitable cases admitted to hospital during the epidemic rise of 1917.

~~From the middle of September to the end of October, cases of dysentery were being admitted to the Casualty Clearing Station daily, and each day two or three cases were selected for special observation. Bacillary dysentery, like typhoid and other microbial infections of the bowel, is a more or less self-limiting disease, differing in that respect from the chronic progressive course which an untreated amoebic infection generally follows. It was important therefore to select cases of different degrees of severity and at different stages of the disease.~~

[In each case clinical notes were taken on admission and the first stool was carried at once to the laboratory where the fresh specimen was examined macroscopically as well as microscopically. In the absence of pathogenic entamoebae, a provisional diagnosis *of bacillary dysentery* was made. Another film was prepared,

4. The bacilli of dysentery subsequently recognized
by the usual serological + biochemical tests.
The carrying out the former tests Garrow's agglutinator (5)
proved to be of great practical value.

fixed, and stained, for a subsequent and more proplonged examination. In addition, a piece of muco-pus was spread for culture on a plate of Macconkey's medium, which consisted

of:-

Agar Powder	3 grms.
(Taurocholate of Soda	0.5 grm.
Lactose	1 grm.
Neutral Red	1 cc. of a 1% solution.
Ag. destil. up to 100 cc.	

For method of fixing and staining films see Appendix I.

Cultural Methods.

The MacConkey plate on which a loopful of muco-pus had been spread was placed in the incubator for twenty-four hours. Any likely colonies were then picked off and sub-cultured overnight on an agar slope. From these sub-cultures, further sub-cultures were made in Durham's tubes containing respectively 1% Glucose, Saccharose, Lactose, Dulcitol, and Mannite, in litmus peptone water. Another test-tube with peptone water was inoculated for the Indol reaction, and a film of the growth was examined for motility.

A sub-culture which produced acid without gas in glucose only, and which showed neither motility nor indol could be regarded as belonging to the Shiga-Kruse group of dysentery bacilli. Where acid without gas was produced in glucose and mannite, and there was non-motility, the organism

was regarded as of the Flexner-Y group. In the latter, the indol reaction was variable.

Agglutination.

In conjunction with the sugar reactions just described, the sub-cultures from suspected colonies were always subjected to an agglutination test. For this purpose three sera were used:-

(a) B.dysenteriae Shiga, having a titre of 1:1000.

(b) B.dysenteriae Y, with a titre of B. Flexner of 1:3000 and
B. dys.Y of 1:7000.

(c) The Polyvalent serum exhibited in the treatment of patients. As a rule, these sera were used in dilutions of 1:200 and only in special cases were tests made to determine the limit of dilution in which agglutination occurred.

Dealing with so many sub-cultures, the ordinary method of mixing the bacillary emulsion and the different sera in small sedimentation tubes in order to carry out the macroscopic test would have involved much labour, but Captain R.P. Garrow⁽⁵⁾ R.A.M.C. has devised and described an instrument which has simplified the work enormously.

For description of Garrow's agglutinometer, see Appendix II.

Differential Count of the Cellular Exudate.

[In each slide, 500 cells were counted and the following table of figures (Table III) gives the results in fifteen

6/18/25
18 cases noted

TABLE III

Details of Cellular Exudate in Acute Bacillary Dysentery
Differential Counts.

turn heads

Case No.	Name	Day of Illness	Type of Bacillus Isolated	Percentage of Polymorph Leucocytes.	Large Mononuclears	Lymphocytes	Eosinophiles	Macrophages	Epithelial Cells	Plasma Cells.
1	Byrne	1st	Flexner Y.	93.6	1.0	2.2	-	1.2	0.8	1.2
2	Battersby	2nd	Shiga.	90.2	0.4	3.2	-	2.0	0.8	3.4
3	Thomas	3rd	Flexner Y.	87.8	1.2	2.8	-	1.8	4.2	2.2
4	Gallagher	4th	Shiga.	94.2	0.6	2.4	-	1.0	0.8	1.0
5	Shepherd	5th	Flexner Y.	94.2	0.6	2.6	-	1.6	0.6	0.4
6	Cook	6th	Shiga	85.4	2.2	1.4	-	6.4	3.8	0.8
7	Williams	7th	Shiga	87.6	6.2	3.2	-	1.2	0.8	1.0
8	Newton	8th	Shiga	89.8	4.0	3.4	-	1.0	1.4	0.4
9	Prest	-	Shiga	92.0	1.8	3.2	-	1.4	0.2	1.4
10	Tindall	-	Shiga	91.6	0.6	2.0	-	1.6	1.4	2.8
11	Chandler	-	Shiga	90.4	0.6	3.8	-	1.6	1.2	2.4
12	Moore	-	Flexner Y.	87.4	1.6	3.2	-	2.2	2.4	3.2
13	Jones	-	Flexner Y.	91.6	0.8	2.4	-	1.2	2.0	2.0
14	Milton	-	Flexner Y.	91.4	0.8	3.6	0.2	1.4	1.4	1.2
15	Ferguson	-	Shiga	93.2	1.8	2.6	-	1.2	0.4	0.8
AVERAGE				90.69	1.61	2.80	0.01	1.80	1.48	1.61

F.Y., Flexner Y. S., Shiga.

18 lines
Solid

TABLE IV.

Details of Cellular Exudate
in Acute Amoebic Dysentery:

(Differential Counts.)

These are the organisms found in active E. histolytica

Case No.	Name.	Organism found.	Percentage of Polymorph Leucocytes.	Large Mononuclears.	Lymphocytes.	Eosinophils.	Macrophages.	Epithelial Cells.	Plasma Cells.	Pyknotic Cells.
1.	MacDonald.	Active E. histolytica.	5.6	0.8	3.4	2.4	-	2.0	3.4	82.4
2.	Gibson.	"	4.8	1.0	2.2	4.4	-	2.2	2.4	83.0
3.	Dynes.	"	5.6	0.2	1.8	2.4	-	1.4	1.6	87.0
4.	Blandford.	"	10.8	0.6	2.4	2.0	-	0.8	0.8	82.6
5.	Houghton.	"	10.6	0.8	2.6	4.8	-	0.2	0.8	80.2
AVERAGE.			7.5	0.7	2.5	3.2		1.3	1.8	83.0

6/10/10

Macrophages, no result.

cases of acute bacillary dysentery. The diagnosis in each case was established by the isolation of colonies which (a) gave the correct sugar reactions, and (b) showed agglutination in the appropriate serum. The first eight cases of the series have been arranged according to the stage of the disease; the last seven were taken in the order of admission. Other bacillary cases were counted, but as the results were all so uniform, it was considered that the fifteen selected would be sufficient to give an average estimate.

In order to emphasize the significance of the figures in the differential diagnosis of bacillary dysentery, I have drawn out a contrast table (Table IV) to show the average cellular content of an amoebic stool. Five cases were selected at random, in all of which the Entamoeba histolytica was found in the active vegetative form, and in none of which were any dysentery bacilli recovered from cultures. The five cases are placed in the order of admission, and in this table a further column has been added to include the cells undergoing pyknosis. In each slide, 500 cells were again counted, and a percentage struck.

A comparison of Tables III and IV brings out the following salient features:-

6/16
Old. par.
Bacillary Dysentery.

Amoebic Dysentery

1. The preponderance of polymorph leucocytes, which constitute on an average 90% of the total cellular exudate.

2. Eosinophile cells were seldom or never present.

3. Macrophages were present in limited numbers averaging about 2%, but their size renders them very conspicuous.

4. "Pyknotic" bodies were not found in any of the fifteen cases under review.

1. The small proportion of polymorph leucocytes, the average content being only 7½%.

2. The presence of Eosinophiles is a conspicuous feature, the number varying from 2% to nearly 5%.

3. Macrophages were not found in any of the five cases of this series.

4. "Pyknotic" cell remnants were present in all cases, their average number reaching the very high proportion of 83%.

Spt

The characteristic features noted in juxtaposition seem to establish a very clear line of demarkation between the cellular exudates of Amoebic and Bacillary Dysentery, and yet since the early stages of the war in the East, much discussion of a highly controversial nature has centred round this very point. Cytological experts working in different laboratories have issued reports that could not be reconciled or explained on the basis of the personal equation. After studying the effect of varying conditions on the faecal constituents in a number of cases I am of opinion that the whole problem can be narrowed down to an examination of two points.

- I. The proximity of the laboratory to the active troops.
- II. The freshness of the faecal specimen.

I. If a laboratory is situated at the base, and, as not infrequently happens, cases do not reach the base hospital until their tenth or twelfth day of illness, the bacteriological records must differ very widely from those of a laboratory behind the front line where specimens can be examined possibly on the same day as the man first appears on "sick parade". It has been stated that bacillary dysentery is a self-limiting disease, and whether treatment has been rigidly carried out or not, the clinical and microscopical picture has generally undergone fundamental changes in ten or twelve days.

This point might be illustrated by a resume of my notes on Case No. I of Table X.

Pte. Byrne W. was admitted to hospital on Oct. 18th 1917 suffering from slight pyrexia. As he had previously been subject to attacks of ague, his card bore a diagnosis of Recurrent Malaria. A blood film was taken and no Malarial parasites were found. During the night diarrhoea commenced and by dawn, the 19th he was in great discomfort and passing scanty stools every hour. The tongue showed a thin white film; there was considerable tenderness in the left iliac region and the sigmoid was palpable.

Stool.(a) 19-10-17:-

Macroscopically - a scanty "mucus" stool of pearly appearance and streaked with blood.

Microscopically - Red blood corpuscles, numerous leucocytes, some macrophages, and columnar epithelium. No Amoebae were found.

Cultural - A piece of the mucus-pus was spread on a MacConkey plate and incubated for 24 hours. A good growth resulted and roughly half the colonies on the plate were found to be of the B.Flexner-Y type. The only therapeutic agent employed was Sod. Sulph. 37 every 4 hours, in solution.

20-10-17:-

There was less discomfort but he passed 15 stools in the 24 hours, and the sigmoid was more palpable.

Stool.(b) 21-10-17.

Macroscopically - Yellowish-grey "mucus" stool, flecked with blood.

Notes on Case No. I. of Table X. continued

Stool (b) 21-10-17.

Microscopically - Numerous pus cells and red blood cells, but the pus cells were distended and more refractile; macrophages were present in small numbers; very few epithelial cells.

Cultural - There was a good growth of different organisms but only five colonies had the appearance of B. dysenteriae. These five colonies were subcultured and tested by agglutination and the sugars, but only one (a,B, Flexner-Y) responded to all the reactions.

22-10-17.

Patient was less distressed, and had longer periods of rest.

Stool(c) 23-10-17.

Macroscopical - Scanty stool of very tough grey, mucco-pus.

Microscopical - Pus cells, with degenerate nuclei; very few red blood corpuscles; macrophages plentiful.

Cultural - The MacConkey plate showed many colonies of acid-forming organisms but only five were picked out as resembling B. Dysenteriae.

Agglutination and sugar tests again reduced that number to one definite growth of B. Flexner-Y.

24-10-17

Generally much improved and not now disturbed at night

Stool (d) 25-10-17.

Macroscopical - Semi-formed faecal stool of healthy colour.

Microscopical - Alimentary debris, but no red blood cells, pus cells, macrophages, or epithelium found.

Cultural - No specific organisms isolated.

This case serves as a type of the class which constitutes probably the bulk of patients invalidated with bacillary dysentery. It was an attack of moderate severity treated simply by rest, dietetic measures, and intestinal flushing, and the stage of convalescence had been reached by the end of seven days. Had this man been passed on through Medical units which were not in touch with a laboratory, he would probably have reached the base at a stage when it would have been impossible to establish a definite diagnosis.

On the other hand, Amoebiasis, unless specifically treated, is notoriously chronic and progressive. The onset is slow and insidious and for the first few days an examination of the stool in an advanced laboratory may reveal nothing definite. By the time the patient reaches the base, if no emetin has been administered in the meantime, the symptoms are usually well-marked, and the pathogenic Amoebae are found without much difficulty. It is not surprising therefore to find variations in the records of different laboratories.

11.2 The freshness of the faecal specimen bears a very close relationship to the proportion of positive bacteriological findings compared with the total number of stools examined. *Ceteris paribus*, the percentage of positive findings reaches its maximum when the laboratory is attached to an advanced field unit, and the bacteriologist can select his specimen directly from the bedpan within half an hour after the stool has been passed.

In many instances, owing to military exigencies, field medical units were situated at some distance from the laboratory, and specimens had to be transported in small glass faeces tubes. Under such conditions, the results were unsatisfactory in direct proportion to the time occupied on the journey. It was a common experience, for instance to find in an absolutely fresh stool abundant active pathogenic Amoebae; when the same specimen had been standing for several hours, an examination often revealed no trace of the protozoa. Portions of stool containing vegetative *Entamoeba histolytica* were placed in faeces tubes and kept in the incubator; others were kept in the ice-chest and at the end of three hours I was unable to find Amoebae in any of them. The organisms disintegrate and disappear very rapidly after leaving the intestine. The "Bacillary" stool also undergoes deterioration from a bacteriological point of view, though the process is not quite so rapid as in the "Amoebic" type.

[In order to determine the rate at which dysenteric bacilli disappear from the faecal specimen, I carried out a series of experiments with some typical stools, and came to the conclusion it was rarely possible to recover, by cultural methods, the specific bacilli from a motion that had been passed eight hours previously. The following extracts from my notes on an experimental case will serve to illustrate the methods adopted and the results obtained.

Bombr. G. was admitted to the C.C.S. on Oct. 22nd 1917 suffering from acute dysenteric symptoms. The illness had commenced two days previously. On admission his face was flushed; his temperature was 99°F. and he was passing about thirty scanty motions in the 24 hours. He complained of spasmodic pains across the lower abdomen; there was tenderness in the left iliac region and the sigmoid was palpable but soft.

Stool.

Macroscopical - Consisted of a small quantity of blood-streaked mucus which adhered to the bedpan.

Microscopical - Numerous red cells and pus cells, a few macrophages and blastocysts.

Cultural - Numerous colonies which proved to be glucose- and mannite-fermenters and were agglutinated by the high titre serum for B. Flexner-Y.

The following day the symptoms were still severe and and he was passing the same type of "blood and mucus" stool about every hour.

A characteristic motion which was passed at 9.30 a.m. was carried to the laboratory, and at 10 a.m. a small piece was spread on MacConkey's Medium and placed in the incubator. The rest of the motion was left in the bedpan under the ordinary conditions obtaining in the laboratory and at 2 p.m. a second piece was spread on a MacConkey's plate, and incubated. A third plate was spread at 6 p.m. and a fourth on the following day at 10 a.m., 24 hours after the exudate had left the intestine.

The plates were all left in the incubator for 24 hours and then examined with the following results:-

(1) Plate spread at 10 a.m. 22-10-17.

Five-sixths of the colonies on the plate had the small greenish dew-drop-like appearance of the growing *B. dysenteriae* and on subculture these were proved to be of the *B. Flexner Y* type. The other sixth, consisting of 55 colonies, all gave the reactions of *B. Acidi Lactici*.

(2) Plate spread at 2 p.m. 22-10-17.

The growth was much heavier than in the first plate and was mixed.

- (a) Ten colonies were found which on subculture proved to be of the *Flexner Y* type.
- (b) Numerous colonies of *B. Acidi Lactici*.
- (c) A considerable number of whitish colonies with bluish opalescence. These were not agglutinated by the sera for *B. Shiga* or *Flexner Y*, but responded to the sugar and chemical tests for the *Tor* groups of *B. dysenteriae*.
- (d) Some opaque red colonies belonging to the *B. Paracolony* group.

(3) Plate spread at 6 p.m. 22-10-17.

No colonies of *B. Flexner Y* could be discovered but there was a heavy growth of *B. Acidi Lactici*, and some luxuriant white colonies having the appearance of spots of diluted milk, which proved on subculture to be a strain of *Bac. Proteus*.

(4) Plate spread at 10 a.m. 23:10-17

No colonies of *B. Flexner Y* were found. There was a luxuriant flora of different organisms in confluent colonies, none of which had any importance as factors in the dysenteric symptoms.

In this case, the culture taken from an absolutely fresh stool, showed an almost pure culture of B. dysenteriae (Flexner Y.) After standing 4 hours the growth of B. Flexner Y. was scanty and other organisms were in the ascendancy. After standing for 8 hours the dysenteric organisms were completely overgrown and it was impossible to isolate the specific bacilli. If circumstances had prevented a cultural examination being made within 8 hours of defaecation, the result would have been negative.

The findings in similar laboratory experiments carried out at the same period need not be detailed, as they presented very uniform records. [The practical side of the question can be demonstrated by a few figures taken from the returns of the laboratory with which I was connected in 1918. Here the specimens were sent in from various medical units, most of which were situated at some distance away. Portions of the stools were placed in faeces tubes, and sent to the laboratory by post or by messenger, and frequently an interval of from 6 to 24 hours had elapsed before the examination was made. Out of 900 specimens examined by culture during the year, only 75 or 8.3 % gave definitely positive results. Of these 75, organisms of B. Shiga type were isolated in 58 cases, and B. Flexner Y. in the remaining 17. ~~Compare these~~

should be compared
The results with the findings of the laboratory during the autumnal incidence of dysentery in 1917 (vide table). During that period the conditions for successful culture were ideal because the bacteriological camp was adjacent to the Casualty Clearing Station and bedpan specimens were carried to the laboratory immediately they were passed. Out of 194 specimens cultured under such conditions, 116 or 60% gave positive results. Of that number, 56 were of B. Shiga type and the remaining 60 were B. Flexner Y.

Summary Conclusions } *small table*

spt [In conclusion, as the result of personal experience of epidemics of dysentery in the field, I would lay emphasis on the following points.

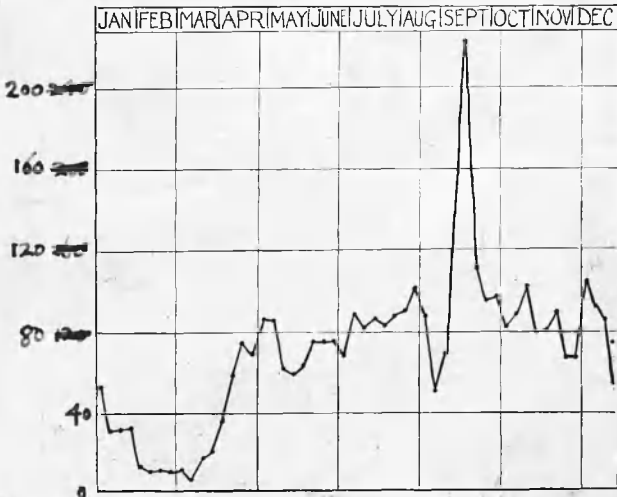
1. The clinical^{ian} and the pathologic^{ist} should be as constantly associated as the surgeon and the anaesthetist.
2. The laboratory should be in close proximity to the source of material.
3. Considering the vicissitudes which surround the isolation of specific dysentery bacilli, much more stress should be laid on a diagnosis founded upon the characteristic cellular exudate. The most important factor in the treatment of acute dysenteric conditions is time, and as a provisional diagnosis by this method can be made at least twenty-four hours before the bacteriological report is ready, the advantage to the patient is incalculable.

See in reference from p. 37 (6 p.)

2. Remove the plate
for section. Mammis
Top Dis

Anderson

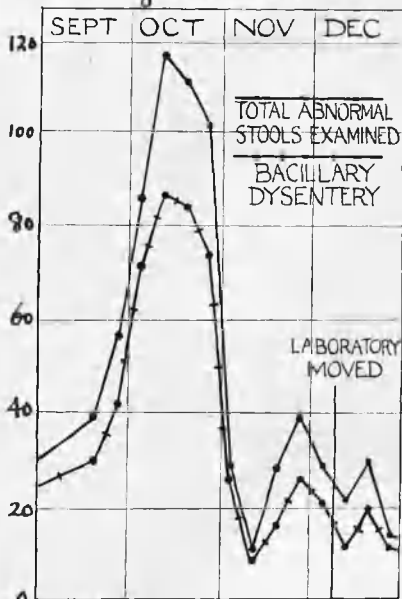
Incidence of Dysentery in 1916
Weekly rate per 100,000 strength



[Chart 1.]

Anderson

Dysentery: Epidemic Rise
in 1917. From one casualty
clearing station. Figures
per week



~~Date~~
~~1917~~

Chart 2. -



FÆCAL FILM FROM CASE OF ACUTE BACILLARY DYSENTERY

- A POLYMORPHONUCLEAR LEUCOCYTE
- B MONONUCLEAR LEUCOCYTE
- C RED BLOOD CELL
- D MUCUS

A

979

April 6, 1921

Dr. Joan Anderson,

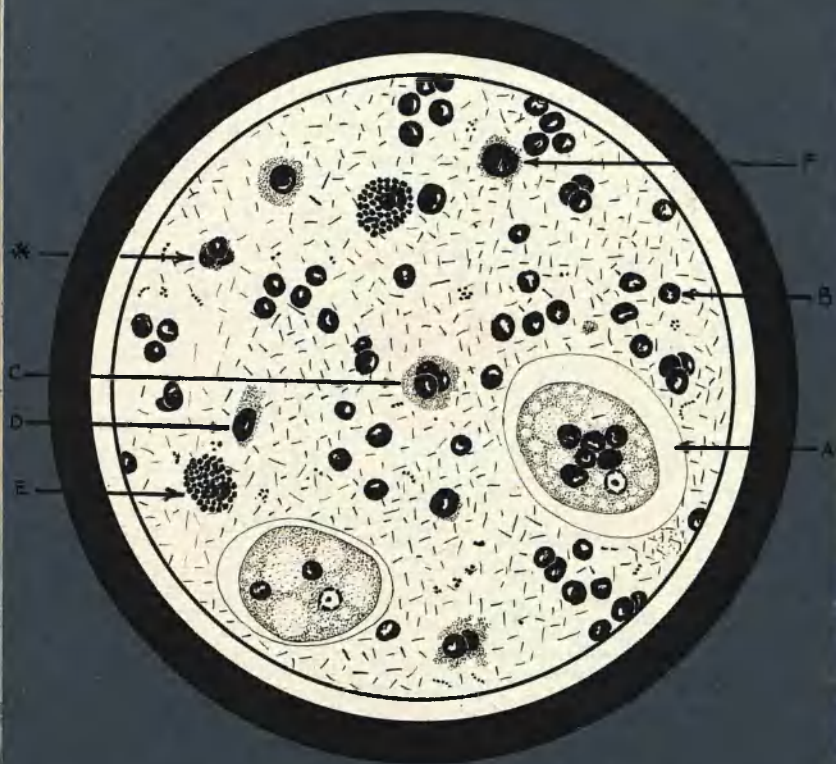
Dear Sir,

On going further into the question of reproducing your Study of Dysentery in the Field, we have not got over the difficulty in allotting so much space both to text, tables, and illustrations. We think it might be more useful to our readers if you were to concentrate on the figures showing the relative frequency of amoebic and bacillary forms of dysentery. The detailed discussions on cells might be cut down where they cover the ground dealt with by Willmore in The Lancet of 1918, vol. II, page 200; and the technique of staining films might also be omitted on the ground of familiarity. Probably some of the drawings have already been sufficiently covered in previous papers. Possibly the best solution would be, if you would like to assume agreement and concentrate on such differences from previous writers as you would like to bring out.

Yours very faithfully,

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FAECAL FILM FROM CASE OF ACUTE AMOEBIC DYSENTERY

- A ENTAMOEBIA HISTOLYTICA
- B RED BLOOD CELL
- C POLYMORPHONUCLEAR LEUCOCYTE * SHOWING PHAGOCYTOSIS
- D MUCOSAL CELL
- E EOSINOPHILE LEUCOCYTE
- F MONONUCLEAR LEUCOCYTE

APPENDIX I

METHOD OF FIXING AND STAINING PAECAL FILMS.

Fixing. The films were fixed by a modification of Schandinn's method.

- (a) The film, while still wet, was placed in a solution consisting of:-

Saturated Sol. of Corrosive Sublimate in

Normal Saline - - - - - 60 cc.

Absolute Alcohol + + + 32 cc.

Glacial Acetic Acid --- 4 cc. (added immediately before use)

Fixed for 10 minutes.

- (b) Washed off with 30% Alcohol, then with weak Iodine solution, and again with 30% Alcohol.
(c) Transferred to 50% Alcohol, then to 70%, then to 90%.
(d) Placed in absolute Alcohol for 15 to 30 minutes.

Straining. Several different methods were tried, until I learned to place most reliance on a combination method suggested by Capt. J.G. Willmore⁽⁴⁾ R.A.M.C., and termed by him the "W" stain. The procedure was as follows:-

- (a) Placed the fixed film in a mordant composed of

Iron Alum - 4% Solution in water - 1 part.

Alcohol -50% 4 parts.

and left in incubator for one hour.

- (b) Transferred to a solution containing
Heidenhain's Haematoxylin stain - - 1 part.
Alcohol - 50% 9 parts.
Stained for one hour in incubator.
- (c) Decolorized almost completely in Acid Alcohol
(Hydrochloric Acid, 1cc., Alcohol (70%), 99 cc.)
until the nuclei appeared as faint shadows.
Washed off with 50% Spirit containing a few drops
of Ammonia, and then with 50% Spirit.
- (d) Transferred to a solution composed of:-
Borrel's Blue - - - 1 cc.
Gauducheau's stain 4 cc.
Distilled Water - - 5 cc.
Stained for 12 to 24 hours.
- (e) Cleared in Acetic-acid-alcohol (Acetic acid .2%
in water, 1 cc. 70% Spirit, 9 cc.). Controlled
under microscope.
- (f) Transferred to a solution of Eosin (.5% in Abs.
Alcohol) for 2-3 minutes, washed off with 90% Spirit.
- (g) Absolute Alcohol - two changes.
- (h) Cleared in oil of cloves, controlling under the
microscope.
- (K) Removed oil with Xylol, and mounted in Canada Balsam.

The advantage of the above method is that it combines the brownish-black Haematoxylin colouring of the chromatin with the polychrome blues and reds of the Romanowsky stains. With films prepared in this way, a differential count of the cellular exudate was then made. In each slide, 500 cells were counted, a percentage struck, and the result recorded beside the clinical and bacteriological notes.

APPENDIX II

Method of carrying out Agglutination devised by

Capt. R.P. Garrow^(S) R.A.M.C.

The agglutinometer consists of a box about 16"x 8" x 8" with a spindle passing through the long axis and prolonged into a handle on the right-hand side. To this spindle is attached a block of wood one face of which is cut to form a bed for the agglutination plate. The plate is the essential part of the device and consists of a piece of thick plate glass measuring 12" long by 1½" broad. The glass is ruled across its breadth so as to divide the plate with 30 sections arranged like a row of microscopic slides and numbered 1 to 30. Each section measures 1½" x ¼". The sections are separated from each other by two parallel grooves which prevent the fluid placed in one section from coalescing with that in another.

The dilutions of the sera are made on a block of hard paraffin or porcelain which has been pitted so as to form three parallel rows each of six indentations. With a glass pipette drawn out to a capillary point, 5 drops of normal saline are counted into each pit. Into the first pit of the first row, 5 drops of the high-titre serum for *B. dysenteriae*

Shiga already diluted say 1:50 are placed. This gives 10 drops of a 1:100 dilution. Five are transferred from the first pit to the second pit of the same row, and the result is a dilution of 1:200. The process is repeated along the row, so that in the sixth pit there is a dilution of 1:3200.

Into the first pit of the second row, 5 drops of the high-titre Serum for *B. dysenteriae* Flexner Y are counted, and the same process is continued along the second row. To the first pit of the third row, 5 drops of Polyvalent Serum for *B. dysenteriae* are added and the process again continued to the end of the row.

From the first pit, one drop is now transferred to the first section of the glass plate. From the second pit, a drop is placed on the second section, and so on, until all the dilutions on the block are represented by a drop on a separate section of the plate.

An emulsion of the sub-culture to be tested is now prepared, and one drop is placed beside the drop of diluted serum on each section of the glass. The plate is slowly rocked about until each pair of drops is well mixed. It is then fixed on the wooden block attached to the spindle of the box, the lid is closed, and the handle is turned for three minutes. By that time, any agglutination is quite visible with the help of a watch-maker's lens, and the different degrees of dilution in which it has occurred are easily read off. By limiting the dilutions to one for each of the three sera, as many as ten sub-cultures can be tested on the one

plate, and as the whole process only occupies several minutes, the saving of time and labour is obvious.

Omit

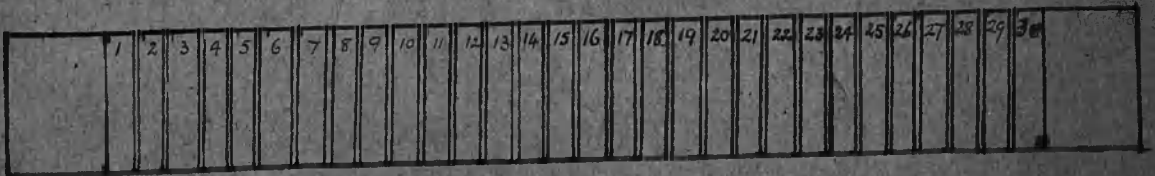


Diagram of glass plate used in Garrod's agglutinating instrument.
Scale - 1/2 size.

6/11

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2. Hiss.	Jour. Med. Res. XIII 1904 No.1. fig. 1.
3. Wenyon & O'Connor	Human Intestinal Protozoa in the Near East. 1917.
4. Willmore & Shearman	<i>The</i> Lancet. 1918 II. fig. 200.
5. Garrow.	Lancet. 1917. I. fig. 262.

