ACUTE BACILLARY DYSENTERY IN KHARTOUM PROVINCE, SUDAN, WITH SPECIAL REFERENCE TO BACTERIOPHAGE TREATMENT: BACTERIOLOGICAL INVESTIGATION.

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(With 4 Charts.)

I. Introduction.

In the autumn of 1927 the writer's attention was attracted by the possibilities of treating acute bacillary dysentery with bacteriophage.

Prof. d'Herelle very kindly supplied a quantity of his bacteriophage for use in bacillary dysentery.

Arrangements were made with Dr Squires, Director of the Khartoum Civil Hospital, and Mr O'Shaugnessy, Medical Inspector, of the Omdurman Civil Hospital, for a combined clinical and bacteriological investigation of bacteriophage treatment in acute bacillary dysentery.

In all cases, the bacteriophage was administered by mouth, and this investigation is concerned only with patients treated in hospital.

Most of the previous studies on clinical bacteriophagy in bacillary dysentery have been concerned with purely clinical criteria of cure. As the bacteriophage acts presumably on the bacillus causing the dysentery, the writer considered that interesting information might be obtained by continued observations on the bacteriology of the stools during treatment.

II. Clinical bacteriology.

(*a*) *Outline of scheme.*

The original scheme was to observe 100 hospital cases of acute bacillary dysentery, 50 of which were treated with bacteriophage and 50 by other methods.

Unfortunately as a result of the low incidence of bacillary dysentery in Khartoum Province, only 60 cases were observed during the two-year-period of the investigation.

The scope of the clinical investigation will be apparent from a study of Table I and the bacteriological from Table II.

Table I. *Clinical.*

Group type: Sudanese.
Number: O.C.H. 3. Name: Osman Aren. Age: 20. Sex: male.
Previous history with special reference to dysentery: nil.
Present symptoms and their duration before coming under observation: passage of blood and mucus in frequent stools for 1 day.

Day ...	1	2	3	4	5	6
Stools	20. xi. 29	21. xi. 29	22. xi. 29	23. xi. 29	24. xi. 29	25. xi. 29
No. per day	15	14	12	13	8	6
Consistency	Liquid	Liquid	Liquid	Liquid	Liquid	Mucous
Blood (macroscopic)	+ + +	+ + +	+ +	+ +	+	+
Mucus (macroscopic)	+ + +	+ + +	+ + +	+ + +	+ +	+ +
Maximum temp. during 24 hours	99	98·4	98	98	98·4	98·4

Treatment—Before admission: none

In hospital: 20. xi. 1929 2 ampoules bacteriophage
21. xi. 1929 1 ampoule „
22. xi. 1929 1 „ „

Bacteriological report on stools.

1st day specimen	20. xi. 1929	Flexner Y
4th „ „	24. xi. 1929	„
8th „ „	27. xi. 1929	„
15th „ „	2. xii. 1929	„
	7. xii. 1929	No *B. dysenteriae* isolated

Table II. *Bacteriological.*

Number: O.C.H. 3. Name: Osman Aren. Age: 20. Sex: male.

Day ...	1	5	8	13	15
Stool	20. xi. 29	23. xi. 29	27. xi. 29	31. xi. 29	3. xii. 29
Consistency	Fluid	Fluid	Fluid	Solid	Solid
Reaction	Alkaline	Alkaline	Alkaline	Alkaline	Acid
Blood	+ + + +	+ + +	+ + +	None	None
Mucus	+ +	+ + + +	+ + + +	+ (on surface)	+ (on surface)
Macrophages	+ +	+	+ +	None	None
Leucocytes	+ + + +	+ (debris)	+ + (debris)	+ (debris)	+
Amoebae	None	None	None	None	None
B. dysenteriae	Flexner Y	Flexner Y	Flexner Y	Flexner Y	None
Non-lactose fermenters ...	+ +	+ + + +	+ +	+	+ (*B. morgan* I)

Identification of dysentery bacillus.

Sugar fermentation.

Date	Glucose	Maltose	Saccharose	Mannite	Dextrin	Indol
20. xi. 1929	Acid	Nil	Nil	Acid	Nil	None formed (4 days)
27. xi. 1929	„	„	„	„	„	„ „
2. xii. 1929	„	„	„	„	„	„ „

Serological type.

20. xi. 1929. Agglutination $\frac{1}{5000}$, with Flexner Y serum.
27. xi. 1929. „ $\frac{1}{2500}$, $\frac{1}{5000}$ (incomplete).
2. xi. 1929. „ $\frac{1}{5000}$.

Action of standard bacteriophage on the bacillus *in vitro*.

20. xi. 1929. Readily bacteriophagable.
24. xi. 1929. „ „
27. xi. 1929. „ „
2. xii. 1929. „ „

Presence of bacteriophage in stools.

2. xii. 1929. None present.
7. xii. 1929. „ „

(*b*) *Technique employed.*

After the patient's admission to hospital a specimen of faeces (day 1 in Table I) was sent to the laboratory before treatment was commenced. Further specimens were obtained on days 4, 8 and 15.

Fresh specimens were always received throughout the investigation, and were plated immediately on receipt.

In the bacteriological examination, a suitable piece of the specimen was selected and spread with a platinum loop on two McConkey plates. After 24 hours' incubation at 37° C., several of the suspicious colonies, which had developed on the plates, were transferred to the sugars.

Preliminary identification of the dysentery bacilli, having been established by means of cultural characteristics, the bacilli were subjected to serological tests.

Serological agglutinations with type sera were carried out using Dreyer's method, but in no case was a Widal reaction with the patient's serum done.

As three type-agglutinating sera only were available, namely, dysentery Shiga, dysentery Flexner, and dysentery Y, obtained from the Institut Sérothérapique et Vaccinal Suisse, Berne, the old classification of the Flexner group was retained for the purpose of this investigation.

In the cytological examination (Table II), in reference to the presence of blood in the stool,

+ + + + = much blood macroscopically,
+ + + = streaks of blood macroscopically,
+ + = no blood macroscopically, but considerable microscopically,
+ = few blood cells microscopically,

the amount of mucus was estimated macroscopically and the leucocytes (pus cells) and macrophages microscopically.

After complete identification by means of cultural, biochemical, and serological characteristics, the causal dysentery bacillus was submitted to the action of the bacteriophage *in vitro*.

Clearing of mixtures of the bacteriophage and suspensions made by adding young agar cultures of the bacillus to broth, was looked for after 24, 48, and 72 hours' incubation at 37° C. Sub-cultures on agar were made from the broth mixture at the end of each 24-hour interval and the formation of "plaques" in these sub-cultures were noted.

In the charts which follow:

Clear broth at end of 24 hours and sterile agar sub-culture = readily bacteriophagable (+ +).

Incomplete clearing of broth in 24 hours, but the formation of "plaques" in agar sub-cultures = bacteriophagable (+).

No clearing of broth in 72 hours and no formation of "plaques" in agar sub-cultures = not bacteriophagable with the standard bacteriophage (0).

(c) *Results.*

Of acute bacillary dysentery 60 cases were investigated, 31 being British and 29 being Sudanese.

The types encountered in these 60 cases will be found in Table III.

Table III. *The number of dysentery cases investigated.*

A = bacillus bacteriophagable *in vitro*. B = bacillus *not* bacteriophagable *in vitro*.

Type	Remarks	British			Sudanese		
			A	B		A	B
Shiga	Showing typical morphology and cultural characteristics, and being agglutinable by Shiga serum	2	2	—	13	13	—
Flexner	Showing typical morphology and cultural characteristics, and being agglutinable by Flexner serum	4	4	—	1	1	—
Flexner Y	Showing typical morphology and cultural characteristics, and being agglutinated by Y serum	11	10	1	12	11	1
Sonne (probable)	Showing typical morphology and cultural characteristics, and not agglutinated by Flexner and Y serum. No serological classification possible	2	2	—	1	1	—
Schmitz (probable)	Showing typical morphology and cultural characteristics but not agglutinated by Shiga serum	3	—	3	Nil	—	—
Flexner	Showing typical morphology and cultural characteristics of Flexner group, but not agglutinable by Flexner or Flexner Y serum	9	5	4	2	—	2

Out of the 60 strains of dysentery bacilli isolated, only 12 failed to show evidence of bacteriophagy *in vitro* with the standard bacteriophage.

In the 43 strains of *B. dysenteriae* confirmed serologically, only 2 showed no evidence of bacteriophagy with the bacteriophage.

It was only possible to follow 48 patients throughout their illness, and the course of the disease in these cases is tabulated in Charts I–IV.

Type of causal bacillus proved serologically:
- Chart I = Sudanese cases (Shiga).
- Chart II = Sudanese cases (Flexner Y).
- Chart III = British cases (Shiga, Flexner and Flexner Y).

Type of casual bacillus not proved serologically:
- Chart IV = British and Sudanese cases (Flexner group, Sonne and Schmitz).

The control cases were unselected, and the writer was not aware of the type of treatment employed until the investigation was complete.

Explanation of Charts.

K.C.H. = Khartoum Civil Hospital case.

O.C.H. = Omdurman Civil Hospital case.

Column A = amount of bacteriophage in cubic centimetres given by mouth (bacteriophage treatment being started on the first day).

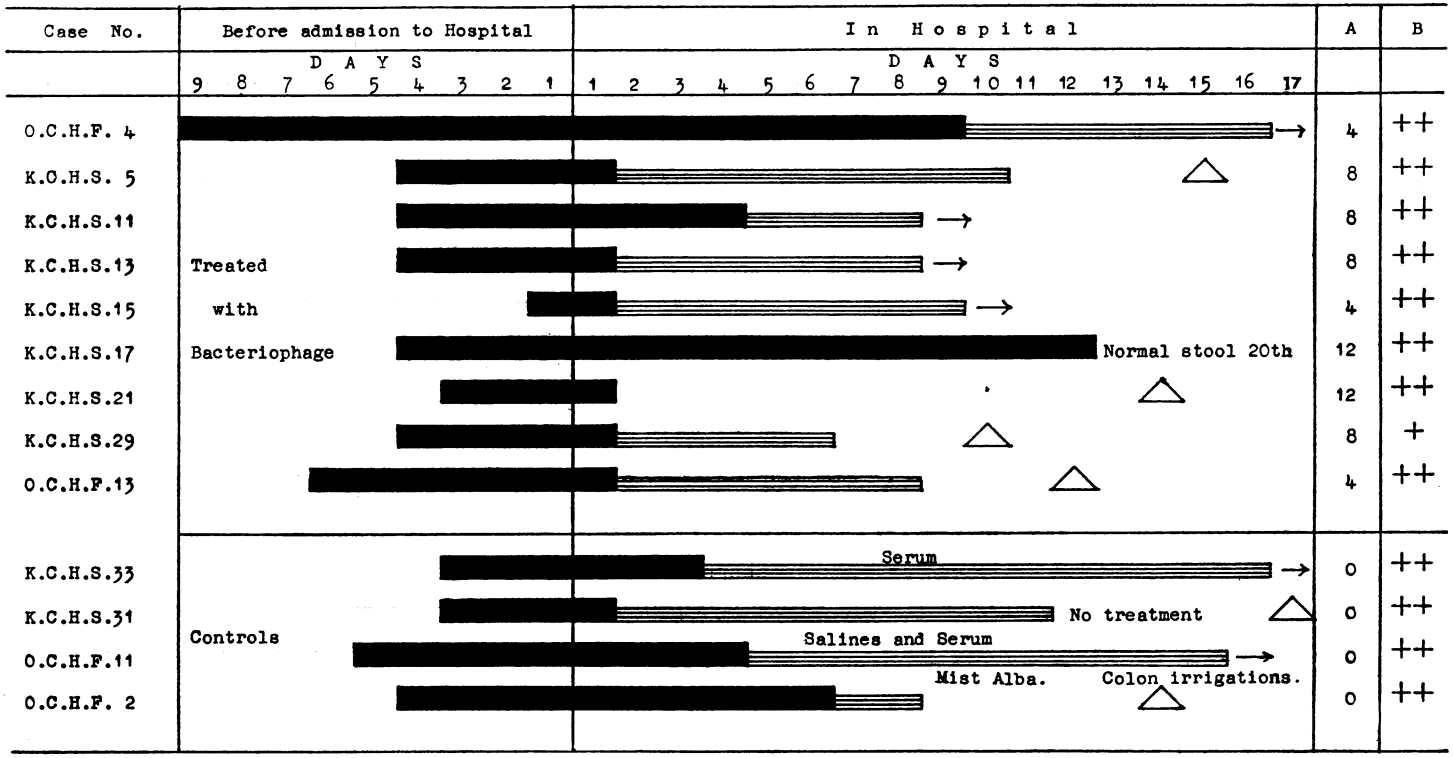


Chart I. Sudanese cases (Shiga).

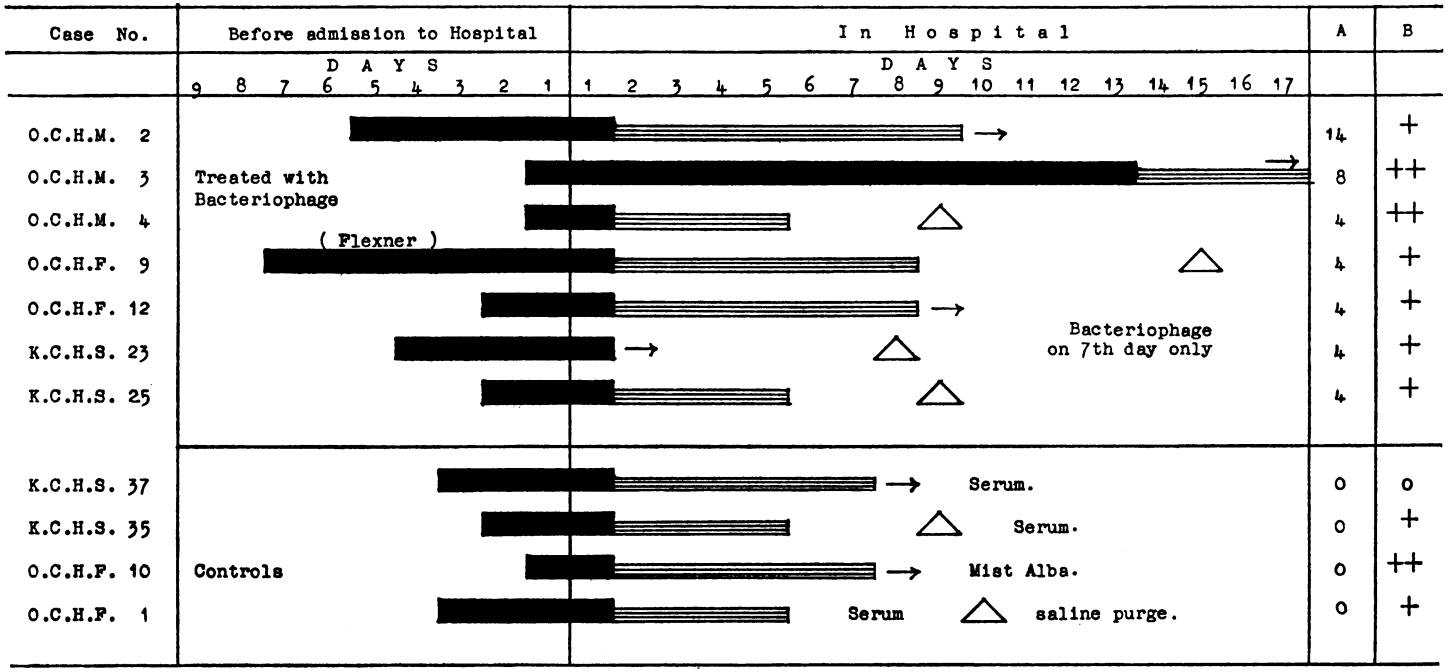


Chart II. Sudanese cases (Flexner Y).

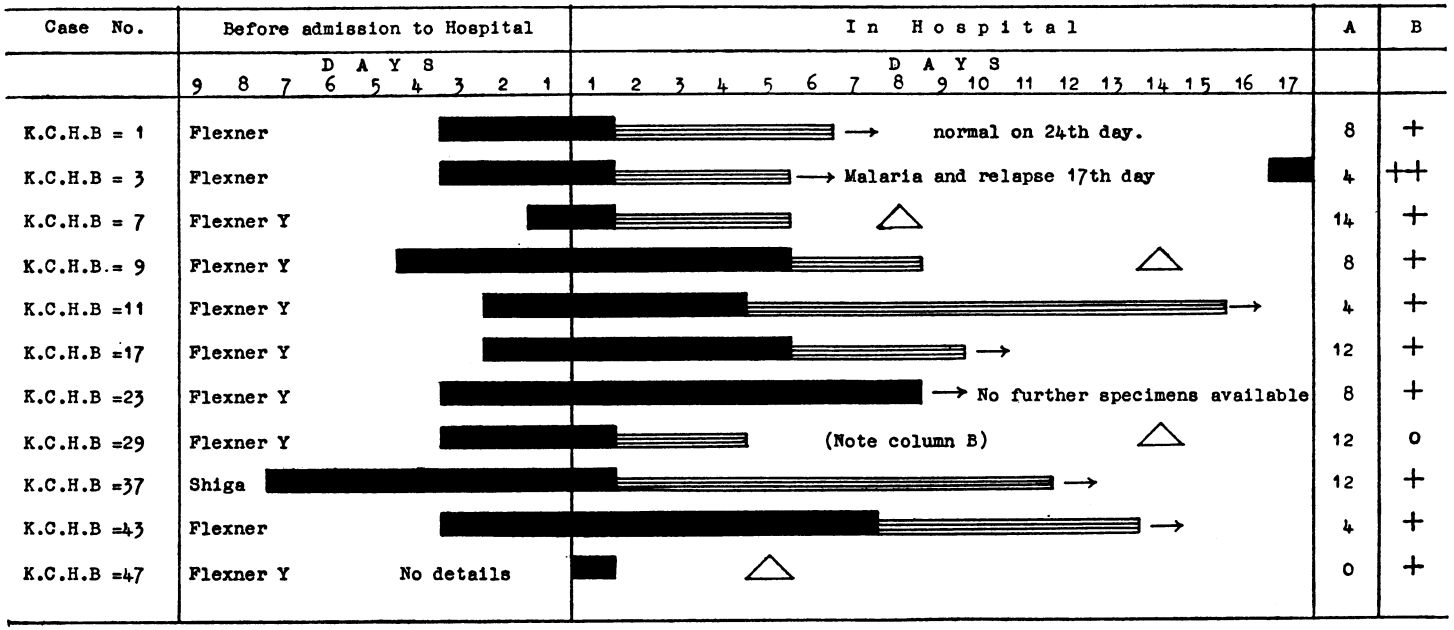


Chart III. British cases (Shiga, Flexner, Flexner Y).

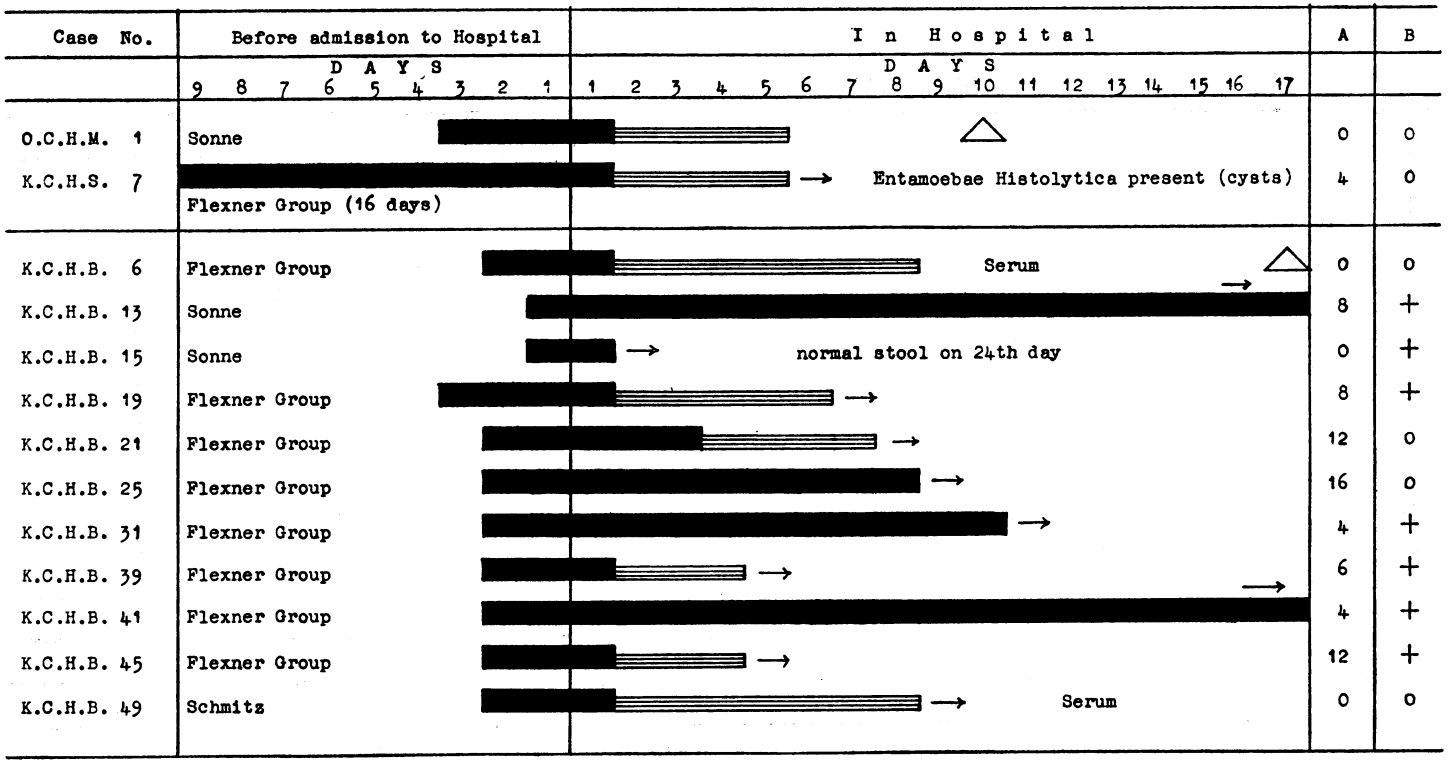
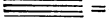


Chart IV. British and Sudanese cases (mixed types).

Column B = action of the bacteriophage on the causal bacillus *in vitro*.

+ + = readily bacteriophagable.
+ = bacteriophagable
0 = not bacteriophagable.

 = bacteriologically positive, up to the last day on which a positive result was obtained.

 = cytologically positive (blood, mucus, pus) up to the last day on which a positive result was obtained.

△ = normal stool passed.

→ = still passing mucus and pus on last day of examination, no further specimens being available.

N.B. Although cases may have been bacteriologically positive on days 2 and 3, and negative on day 4, only the positive finding on day 1 is charted.

Study of the charts will show that the patient's progress was not influenced apparently by either the period of illness before admission to hospital, the type of treatment employed, or whether the bacillus was bacteriophagable *in vitro* or not.

There were no fatal cases in the series, and no dramatic results due to the bacteriophage treatment were demonstrated.

III. POSSIBLE FACTORS INFLUENCING BACTERIOPHAGY *IN VIVO*.

The results obtained in the clinical investigation show that the bacteriophage, which acts so well *in vitro*, does not have a similar action *in vivo*.

As the bacteriophage in the human body may be influenced by many factors, such as the acidity of the gastric juice, the length of time it remains in the alimentary tract, the presence of blood and mucus in the intestine in dysentery, and resistant forms of the dysentery bacilli, it seemed important to estimate the influence of these factors.

In carrying out the following experiments certain details were observed:

1. The bacteriophage employed was taken from ampoules belonging to the same batch as those used for therapeutic purposes.

2. A strain of *Bacillus shiga* and one of *B. flexner* Y were selected for testing purposes. Both strains had been recently isolated from cases of acute bacillary dysentery and gave typical morphological and serological characteristics. Both the testing strains were readily bacteriophagable, giving complete clearing of normal suspensions of 250 million bacilli per cubic centimetre in broth within 24 hours at 37° C. with dilutions of 10^{-11} of the bacteriophage.

3. Suspensions of the bacilli were made only from 24-hour-old agar slope cultures.

4. The reaction of all media and filtrates was kept within a range of *p*H 7·4 to *p*H 8·2.

5. Chamberland L 3 filter candles were used throughout for filtration, all candles used being tested periodically to ascertain whether they would pass the bacteriophage satisfactorily, even when this was present in minute quantities.

(*a*) *The acidity of the gastric secretion.*

200 c.c. of bacteriophage active against *B. shiga* and a similar quantity active against *B. flexner* Y were prepared by adding 0·5 c.c. of the standard bacteriophage to normal suspensions of the bacilli in broth, and filtering after 24 hours' incubation at 37° C.

The filtrates were pipetted into test tubes, 10 c.c. to each, and were submitted to treatment with hydrochloric acid for periods of 5, 10, 15, 20, 30, 45 and 60 minutes.

By the addition of $N/10$ HCl the bacteriophage, which had a *p*H of 8, was adjusted to a *p*H of 3, and at the end of the time interval, the adjustment to *p*H 8 was made immediately, by the addition of $N/10$ NaOH.

The contents of all the tubes were then filtered and tested for the presence of active bacteriophage.

No alteration could be detected in the activity of the bacteriophage, as that treated for 60 minutes with acid was equally as active as the control in dilutions of 10^{-11}.

(*b*) *Intestinal mucus.*

Experiments were carried out, using autoclaved 25 per cent. normal saline emulsions of intestinal mucus obtained from bacillary dysentery cases, instead of broth as the medium for bacteriophagy.

It was found that bacteriophagy was incomplete in mucus at the end of 72 hours at 37° C., although broth controls were rendered sterile in 24 hours.

This result, however, cannot be exactly analogous to the natural process, as it occurs in the human intestine, because the mucus was considerably diluted and subjected to autoclaving, which undoubtedly altered the protein to a considerable extent, and the presence of other intestinal organisms was excluded.

(*c*) *Blood serum.*

The blood serum from 30 normal individuals was pooled, so that an average specimen of human serum was obtained. This serum was passed through a Chamberland L 2 filter candle, but was not inactivated.

Various dilutions of the sterile serum in normal saline, ranging from 5 to 100 per cent. were put up in sterile test tubes so that each tube contained 2 c.c. of fluid.

All tubes were inoculated with equal amounts of either *B. shiga* or *B. flexner* Y giving an initial suspension of 125 million bacilli per c.c. and three drops, each of 0·05 c.c. of the bacteriophage, were added to certain of the tubes. Control inoculated broths were similarly treated.

The tubes were incubated at 37° C. and sub-cultures on agar were made at 24, 48 and 72 hours, after vigorous shaking of the fluid contents of each tube.

It was found that, while the broth controls were rendered sterile in 24 hours, both *B. shiga* and *B. flexner* Y were still living in the 5, 10 and 20 per

cent. serum dilutions at the end of 72 hours in spite of the presence of the bacteriophage.

Dilutions between 50 and 100 per cent. of the serum were sterile at the end of 48 hours, possibly as a result of the bactericidal action of the serum.

Human blood serum in various dilutions thus compares unfavourably with laboratory broth as a medium for the process of bacteriophagy.

(*d*) *Resistant forms of the dysentery bacilli.*

As a result of a few examinations, it has been found that dysentery bacilli, isolated during the course of an attack of acute bacillary dysentery, are as readily bacteriophagable *in vitro* whether isolated on day 1 or day 12 of the disease.

In the case of the patient Osman Aren (Table II) for example *B. flexner* Y isolated on days 4, 8, and 12 in hospital, retained their morphology, cultural characteristics, serological reactions, and remained readily bacteriophagable; being in every respect identical with the bacillus isolated on day 1.

IV. OBSERVATIONS ON THE ORAL ADMINISTRATION OF BACTERIOPHAGE.

(*a*) *In normal individuals.*

The faeces of seven healthy British laboratory workers were tested for the presence of bacteriophage virulent for the test strains of *B. shiga* and *B. flexner* Y.

A faecal emulsion was made by adding 5 grm. of faeces to 50 c.c. of broth. After 24 hours' incubation at 37° C. this emulsion was strained through gauze, passed through a Seitz clarifying disc (Kharschichten), and filtered through a Chamberland L 3 candle.

Each of the subjects was then given two ampoules (4 c.c.) of the bacteriophage, this being the minimum dose used in treating the dysentery cases, and their faeces were again tested for the presence of bacteriophage at the end of 24 and 48 hours.

The method of obtaining the sterile faecal filtrate was controlled by adding 0·05 c.c. of the bacteriophage to a faecal emulsion in which there was no demonstrable bacteriophage, and subjecting this to the same process of filtration as the other faecal emulsions. Very active bacteriophage was demonstrated in the sterile filtrate of this control.

Of the sterile faecal filtrate 4 c.c. were tested in each case, 2 c.c. against *B. shiga* and 2 c.c. against *B. flexner* Y.

In one case a natural bacteriophage was present, weakly active against *B. shiga*. This bacteriophage acquired virulence for *B. flexner* Y after several passages against *B. shiga*.

The faeces from the other six workers were completely negative when tested for bacteriophage.

Assuming that the average weight of the workers was 70 kg., the amount

of bacteriophage was 4 grm., and that it was equally distributed through the body, then 1·0 c.c. of the faecal filtrate should contain a detectable quantity of the bacteriophage.

$$\frac{\text{4 grm. of bacteriophage}}{\text{70,000 grm. weight of individual}} \times \frac{\text{5 grm. of faeces}}{\text{50 grm. of broth}} = 2 \times 10^{-5} \text{(approx.)}.$$

As previously stated, however, it was possible to detect the bacteriophage in dilutions of 10^{-11} and, as the faeces are the home of the bacteriophage, a greater concentration in them might be expected than in the rest of the body.

(*b*) *In cases of acute bacillary dysentery.*

Unfortunately, it was only in the later cases of dysentery that any attempt was made to examine the faeces for the presence of the bacteriophage, as the writer had accepted the statements of d'Herelle concerning the presence of bacteriophage in dysentery stools.

Case O.C.H.M. 4 (Chart II) is of interest, being one of the apparent successes resulting from the administration of the bacteriophage.

This case was ill one day before admission to hospital; on day 1 in hospital the stools were positive for a readily bacteriophagable *B. flexner* Y; four days later, the stools were semi-solid and bacteriologically negative; on day 8 in hospital the patient passed a normal stool.

Of the bacteriophage 4 c.c. were given on day 1 in hospital, but examination of the faeces on day 5 showed no bacteriophage active against the patient's own bacillus, nor against the test strains of *B. shiga*, or *B. flexner* Y. On day 8 in hospital a bacteriophage was present in the faeces, weakly active against *B. shiga*, but even after two passages against *B. shiga*, it had acquired no virulence for the patient's own bacillus nor the test strain of *B. flexner* Y.

Osman Aren O.C.H.M. 3 (Tables I and II) was given 4 c.c. of bacteriophage on day 1 in hospital, 2 c.c. on day 2 and 2 c.c. on day 3.

A readily bacteriophagable *B. flexner* Y persisted in the stools for 12 days, and examination of the faeces on days 12 and 15 failed to show any bacteriophage active against the patient's own bacillus or the test strains of *B. shiga* and *B. flexner* Y.

Amna Ahmed O.C.H.F. 13 (Chart I) was given 4 c.c. of bacteriophage. This patient had been ill for 6 days before admission to hospital. A readily bacteriophagable *B. shiga* was present in the faeces on day 1, but this was not recovered on day 4, and the patient passed a fairly normal stool on day 8.

Although the patient made a rapid recovery in hospital, no bacteriophage active for her own bacillus or the stock *B. shiga* was demonstrated in her faeces on either day 4 or 8.

V. Discussion.

The writer agrees with d'Herelle (1926, p. 62) who states with reference to the phenomenon of bacteriophagy: "how vain it is to attempt from a single experiment to fix the limiting conditions, or the optimal conditions for

the reaction, since they vary for each race of the bacteriophage which acts, and for each bacterial strain subjected to its action."

To anyone familiar with acute bacillary dysentery in its variety of forms—mild catarrhal, ordinary acute, fulminating, choleraic, and relapsing—such a statement as the following dealing with the bacteriophage treatment of bacillary dysentery is frankly absurd. "The results of the treatment of bacillary dysentery with it have been little short of miraculous. In every case, with the solitary exception of a child who was practically moribund when brought to hospital, the bacillary dysentery has cleared up within 24 hours."[1]

If a coagulative necrosis of the intestinal mucosa with exudation of fibrin and polymorphonuclear leucocytes can heal completely in 24 hours, we must revise our knowledge of pathology.

In reference to the experiments carried out with intestinal mucus and blood serum, the work of Handuroy[2] is of interest. He showed while working with several substances such as gums and egg albumin, having the property of augmenting viscosity, that the inhibition of bacteriophagy is a direct expression of the viscosity, quite unrelated to the chemical nature of the viscous substance.

The process of bacteriophagy is modified by normal human blood serum and intestinal mucus, and it is imposible to agree with the statement of d'Herelle (1926, p. 71) that "When added to a bacterium bacteriophage mixture, substances devoid of action upon the bacteria, have in general, no effect upon the phenomenon. Thus the process is not modified for example, by normal serum, ascitic fluid or urine. Bile is unquestionably inhibitory."

Krestownikowa and Gubin[3] found that when the bacteriophage is injected into the body it appears in all tissues within a few minutes, but it is no longer detectable after $6\frac{1}{2}$ to $8\frac{1}{2}$ hours. The experiment dealing with the ingestion of the bacteriophage by normal laboratory workers shows that a similar elimination or destruction takes place when the bacteriophage is ingested.

These findings are contrary to the results obtained by d'Herelle (1926, p. 540) who was able to isolate a bacteriophage from the stools of normal individuals 24 hours after the administration of bacteriophage.

With reference to the presence of a weakly active Shiga bacteriophage in the faeces of Case O.C.H.M. 4 (Chart II), the following statement by d'Herelle (1926, p. 243) is of interest. "Races of the bacteriophage virulent for *B. dysenteriae* Shiga have been isolated up to the present from a variety of sources including the stools of convalescents from bacillary dysentery; the stools of individuals affected with mild intestinal disturbances; the feces of normal men," but it is unlikely that the recovery of this patient was due to the presence of the Shiga bacteriophage in his faeces.

[1] Words in a letter from a correspondent, cited by d'Herelle, Malone and Lahiri (1927) *Trans. VII. Congress Brit. India* (Far Eastern Assoc. Trop. Med.), **2**, 89.

[2] See d'Herelle 1926, p. 64.

[3] Krestownikowa and Gubin (1926), *Centralbl. f. Bakt.* Abt. I, Ref., **82**, 283.

VI. Appendix.

While carrying out this investigation certain interesting points were observed.

(*a*) *The specimens.*

Much has been written on the collection of specimens of faeces in dysentery for bacteriological examination.

Although fresh specimens of faeces were dealt with in this investigation, certain preliminary experiments were carried out to ascertain the interval of time allowable between the passage of a specimen of faeces and plating on McConkey medium in order to get a positive result.

It was not possible to isolate dysentery bacilli from dysentery faeces after 12 hours storage at 37° C. and after 24 hours storage at 22° C.

(*b*) *Concomitant organisms.*

Intestinal disturbance with alkaline stools apparently favours the development of all kinds of non-lactose fermenting bacilli, for abnormal organisms constantly occurred in the later specimens of dysentery faeces (days 4, 8, and 15). Some of these organisms were identified by means of the table in Castellani and Chalmers' *Manual of Tropical Medicine,* others remain nameless.

The commonest of these concomitant organisms in order of frequency were *B. proteus asiaticus, B. alkaligenes faecalis* and *B. morgan* I.

VII. Summary.

1. Two preparations of bacteriophage were used for the treatment of a number of cases of acute bacillary dysentery.

2. The types of *B. dysenteriae* encountered in 60 cases of acute bacillary dysentery are described. In the 60 cases investigated the causal bacillus was bacteriophagable with the standard bacteriophage *in vitro* in 48.

3. The cases are tabulated and no dramatic results due to the bacteriophage treatment are demonstrated.

4. Treatment of the bacteriophage with hydrochloric acid, in a concentration similar to that of the gastric juice, for 60 minutes did not alter the virulence of the bacteriophage.

5. It is shown that 25 per cent. autoclaved intestinal mucus in normal saline, and human blood serum, not inactivated, compare unfavourably with laboratory broth as a medium for bacteriophage action.

6. The results of giving bacteriophage by mouth to normal individuals, and to dysentery patients are described and discussed.

VIII. CONCLUSIONS.

It is probable that the bacteriophage ingested by mouth is quickly eliminated or destroyed by the human body.

The contents of the intestines in dysentery do not appear to be a suitable medium for the process of bacteriophagy.

The clinical course of acute bacillary dysentery is not altered by the oral administration of bacteriophage.

REFERENCE.

D'HERELLE (1926). *The Bacteriophage and its Behaviour.* English transl.

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