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Original Communications.

ON THE VIABILITY AND POSSIBLE VARIATION OF THE *BACILLUS TYPHOSUS*.

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The Viability of the Bacillus typhosus.

The experimental work recorded up to the present has appeared to prove conclusively that the life of the *B. typhosus* in unsterilised soil and water is short. Experiments made in conjunction with Colonel Firth showed that in sewage-polluted soil the typhoid bacillus did not live longer than seventy-four days. Houston's[1] work on the vitality of the *B. typhosus* in raw Thames water demonstrated that 99 per cent. of the typhoid bacilli disappeared during the first week of storage, but a few, specially resistant bacilli, sometimes persisted for several months.

In all these experiments with soil and water *pure* cultures of the *B. typhosus* were added to the various samples of earth and water. Morgan and Harvey, working with the urine of a typhoid carrier, found the duration of life of the *B. typhosus* under natural conditions to be limited to about thirty hours.

Impressed with this result, I determined to repeat their experiments, employing as the infecting material urine and fæces of typhoid carriers and of recent cases of enteric fever. The results of these experiments are given in Table A. It will be seen that under

[1] This paper was written before the publication of Dr. Houston's Sixth Report on Research Work.

TABLE A.

Nature of the experiment and date	Probable number of typhoid bacilli present at the beginning of the experiment	Latest date of recovery and number of bacilli found	Duration of life of *B. typhosus* under the conditions of the experiment	Remarks
I. Sandy soil moistened with urine of Carrier I. May 7, 1909	12,000 per grm. of soil	May 14, 1909, 330 per grm. of soil	7 days ..	Soil allowed to dry at laboratory temperature.
II. Humus from garden moistened with urine of Carrier I. May 7, 1909	6,000 per grm. of soil	May 14, 1909, 40 per grm. of soil	Ditto.. ..	Ditto.
III. Humus from garden moistened with urine of Carrier I. June 4, 1909	1,660 per grm. of soil	June 14, 1909, 280 per grm. of soil	10 days ..	Exposed on verandah; no rain fell; soil quite dry on June 14, 1909.
IV. Stool of Carrier C. added to water. May 18, 1909	At least 50,000 per cc. of water	May 20, 1909, 200 per cc.	2 days ..	Flask kept in the dark; marked multiplication of associated bacteria.
V. Urine of Carrier I. added to tap water. June 22, 1909	At least 2,000 per cc. of water	July 3, 1909, 2 per cc. (574 per cc. on June 23, 2 per cc. on June 30)	11 days ..	Flask exposed to light in the laboratory; marked multiplication of associated bacteria.
VI. Stool of Carrier C. added to tap water. May 23, 1909	At least 100,000 per cc. of water	May 25, 1909, 200 per cc.	2 days ..	—
VII. Urine of convalescent case of enteric fever S. added to tap water. November 10, 1910	70,000 per cc. of water	November 20, 1910, 10,000 per cc.	10 days ..	When this experiment was begun it was thought that the urine contained *B. typhosus*; further study of the microbe showed that it was a form intermediate between *B. typhosus* and *B. coli* (Bacillus S., *see* p. 247).
VIII. Urine of convalescent case of enteric fever S. added to tap water. December 1, 1910	1,000,000 per cc. of water	No typhoid bacilli found on Dec. 8, 1910	Less than 7 days	This urine contained typical typhoid bacilli. Flask exposed to light.

IX. Stool of Carrier C. added to tap water. July 7, 1909	190,000 per cc. of water	Typhoid bacilli not found on July 10, 1909	Less than 3 days	Flask exposed to light.
X. Do.	19,000 per cc. of water	Ditto	Ditto.. ..	Ditto.
XI. Urine of Carrier I. kept in a test tube exposed to light and then added to sterile water. October 1, 1910	100,000 per cc. of water	Feb. 7, 1911, 10,000 per cc.	Still alive (4 months)	When the urine was added to the water it appeared to be a pure culture of *B. typhosus*.
XII. Stool of Carrier C. mixed with garden earth. May 14, 1909	More than 1,000,000 per grm. of mixture	A week later no signs of the *B. typhosus* could be discovered	Less than 7 days	Kept in a dark cupboard.
XIII. Stool of Carrier C. covered with garden earth. May 18, 1909	Ditto	Ditto	Ditto ..	
XIV. Urine of Carrier I. added to sandy soil, which was then exposed to light. May 21, 1909	43,250 per grm. of soil	*B. typhosus* not recovered a week later	Ditto ..	Soil exposed to heavy rain.
XV. Stool of Carrier C. kept in a glass bottle exposed to light. May 18, 1910	10,000,000 per grm. of stool	May 25, 1910, 20,000 per grm. of stool	7 days ..	When passed this stool appeared to contain less than 1,000 *B. coli* per grm.
XVI. Urine of Carrier S. placed in a test-tube plugged with cotton wool and exposed to light. July 2, 1909	12,000 per cc. ..	November 28, 1909, at least 10,000 per cc.	57 days ..	All the urine expended on November 28, 1909.
XVII. Urine of Carrier I. kept as in XVI. May 25, 1909	1,000,000 per cc. ..	April 23, 1910, 1,000,000 per cc.	11 months ..	All the urine expended on April 23, 1909. Reaction of urine neutral.
XVIII. Urine of Carrier I. kept as in XVI. June 26, 1909	At least 1,000,000 per cc.	Sept. 3, 1910, 1,000,000 per cc.	14 months ..	Reaction of urine neutral.

Note.—In Experiments XII. and XIII., the object was to ascertain how long the *B. typhosus* would survive under conditions likely to be met with in the disposal of fæces on the trench system.

these conditions the viability of the *B. typhosus* is much shorter than when pure cultures of the microbe are employed. When typhoid urine and fæces are thoroughly mixed with water or earth the specific bacilli rapidly disappear, and a week after the pollution 99 per cent. of the added bacteria seem to have been destroyed.

The rapid disappearance of typhoid bacilli from typhoid dejecta, whether intimately mixed with earth, or merely covered over as in a trench, or simply deposited on the surface of the ground, is very remarkable, and forms a marked contrast to the prolonged life of the *B. typhosus* in urine kept in flasks. When the urine of a typhoid carrier was kept in flasks exposed to light in the laboratory and frequently opened to the external air, 1,000,000 typhoid bacilli per cubic centimetre were found at the end of fourteen months. It was noted that the specimens of urine in which the typhoid bacilli maintained their existence in practically undiminished numbers showed no change in reaction during the whole of the time the samples were under observation. The specimens contained streptococci, *B. coli*, *B. fluorescens liquefaciens* and *non-liquefaciens*, and a bacillus corresponding very closely to the *B. fæcalis alkaligenes*; these microbes were present in comparatively small numbers as compared with the typhoid bacilli and showed no sign of multiplication in the urine, dilutions above 1,000 always giving practically pure cultures of the *B. typhosus;* yet the moment such a urine was diluted with water an enormous increase of the associated microbes occurred and the typhoid bacilli rapidly disappeared. The following experiment illustrates this point very well: A specimen of urine from a typhoid carrier was plated in various dilutions on bile-salt neutral red lactose agar and found to contain more than 6,000,000 typhoid bacilli and less than 1,000 colon bacilli per cubic centimetre. A sample of water which did not contain *B. coli* in 5 cc. was mixed with this urine in the proportion of one part of urine to five of water, so that each cubic centimetre of the mixture contained over 1,000,000 typhoid bacilli and less than 200 colon bacilli; six days later 6,000,000 colon bacilli per cubic centimetre of the mixture were counted, and at the end of the seventh day they had increased to 7.000,000,000 per cubic centimetre.

The constancy of the cultural characters of the typhoid bacilli isolated from the urine kept in flasks is also worthy of note. The urines were examined weekly, and the typhoid bacilli which lived in one specimen for more than fourteen months gave reactions identical with those obtained at the first examination; they, however, showed a remarkable resistance to the action of the agglu-

tinins present in an antityphoid serum. The following table gives the results obtained :—

Typhoid bacillus	Dilutions of serum 1—50	1—100	1—500	1—1,000
Strain I. isolated after 4 weeks	+	+	+	+
,, ,, ,, 14 months	+	+	0	0
Laboratory strains—				
B. typhosus R and E	+	+	+	+

In view of the feeble reaction of strain I., isolated after fourteen months in urine, and to prove its identity with *B. typhosus*, the culture was injected into a rabbit to see if it would produce agglutinins specific for the typhoid bacillus. The following results were obtained :—

Typhoid bacillus	Dilutions of rabbit serum injected with Strain I. 1—20	1—40	1—100	1—200	1—500	1—1,000
Laboratory strains—						
B. typhosus R. and E.	+	+	+	+	+	±
Homologous strain—						
B. typhosus I. ..	+	±	±	0	0	0

It will be seen that the titre of the serum was higher for the laboratory strains than for the homologous strain.

Major Cummins also examined the rabbit serum for opsonins and reported that it had a marked opsonic reaction for a virulent strain of *B. typhosus*.

As a final test the strain I. was examined as to its power of absorbing agglutinins from a known typhoid serum ; the following results were obtained :—

	Dilutions of serum untreated 1—20	1—50	1—100	1—500	1—1,000
Tested with *B. typhosus* R. ..	+	+	+	+	+
	Dilutions of serum after absorption with Strain I.				
Tested with *B. typhosus* R. ..	0	0	0	0	0

Experiments were then made to determine the virulence of *B. typhosus* excreted in the urine of typhoid carriers I. and S. after the urine had been kept for various periods at the laboratory temperature exposed to light.

Experiment 1.—The urine of carrier I., passed on June 6th, 1909, was plated in January, 1910, and a pure culture of *B. typhosus* recovered. A subculture on agar was prepared and after twenty-four hours' incubation at 37° C., $\frac{1}{10}$ of the growth was injected into the peritoneal cavity of a guinea-pig. Next morning the animal was found dead, and from the peritoneal fluid and spleen a pure culture of *B. typhosus* was isolated.

Experiment 2.—The urine of carrier I., passed on March 25th, 1909, was plated on March 23rd, 1910, and found to contain 1,000,000 typhoid bacilli per cubic centimetre. A subculture from the agar growth of one of the colonies was incubated for twenty-four hours at 37° C., and one standard loopful, weighing 2 milligrammes, was injected into the peritoneal cavity of a guinea-pig. Next morning the animal was found dead and a pure culture of *B. typhosus* was isolated from the peritoneal fluid.

Experiment 3.—The agar slope used in Experiment 2 was kept at the laboratory temperature for three weeks and subcultured. One standard loop, 2 milligrammes, of the culture was injected into the peritoneal cavity of a guinea-pig. The animal remained perfectly well.

Experiment 4.—The urine of carrier S., passed on April 12th, 1910, was plated on April 21st, and found to contain typhoid bacilli. A subculture on agar was made from one of the colonies and one standard loop of the twenty-four hours' growth was injected into the peritoneal cavity of a guinea-pig. The animal was found dead next morning. No typhoid bacilli, however, could be found in the peritoneal fluid, which contained a pure culture of a Gram positive streptococcus giving the following reactions: Glucose, acid, no gas; mannite, acid, no gas; lactose, acid, no gas; dulcite, unchanged; salicin, acid, no gas; cane sugar, acid, no gas; neutral red, unchanged (aerobic conditions); broth, diffuse growth consisting of short chains; gelatine, not liquefied.

[*Note.*—This streptococcus is apparently identical with the one isolated in Experiment II. and during the subsequent intraperitoneal passages. See p. 239.]

Experiment 5.—The culture employed in Experiment 4 was again subcultured on May 7th, and tested in the usual manner; it appeared to be quite pure, no streptococci could be detected. A standard loop of the twenty-four hours' growth was then injected as in the previous experiment. The animal remained perfectly well.

Experiment 6.—The urine of carrier I., passed on June 22nd, 1909, and kept in a test-tube exposed to light in the laboratory for a year, was still found to contain 1,000,000 typhoid bacilli per cubic centimetre. Two colonies isolated from the plate containing $\frac{1}{1000000}$ cc. were planted on agar and labelled I.B. Colony 1 and I.B. Colony 2.

I.B. Colony 1.—One standard loop of a twenty-four hours' subculture on agar was injected into the peritoneal cavity of a guinea-pig. The animal was found dead next morning. From the heart's

blood a pure culture of *B. typhosus* was isolated, but the spleen and peritoneal fluid contained in addition to the *B. typhosus* a motile Gram negative bacillus which gave the following reactions: Glucose, acid and gas; lactose, acid and gas; mannite, acid and gas; dulcite, acid and gas; salicin, unchanged; cane sugar, unchanged; neutral red, gas and fluorescence; broth, no indol; litmus milk, acid, no clot; gelatine, not liquefied.

A broth culture from the original agar slope used in this experiment was carefully examined and found to give all the cultural reactions of *B. typhosus*. The broth culture was then planted on agar and a standard loop of the twenty-four hours' growth was injected into the peritoneal cavity of a fresh guinea-pig. The animal remained perfectly well, and from the peritoneal fluid a pure culture of *B. typhosus* was recovered.

I.B. Colony 2.—The agar growth from this colony was examined in the same manner as colony 1. The guinea-pig which received 2 milligrammes of the twenty-four hours' growth was found dead next morning. From the peritoneal fluid, in addition to *B. typhosus*, a coliform bacillus was isolated; but the cultures from the spleen and heart's blood proved to contain only typhoid bacilli.

Experiment 7.—The urine of carrier I., passed on June 22nd, 1909, and preserved in a test-tube until September 3rd, 1910, was still found to contain 1,000,000 typhoid bacilli per cubic centimetre. On September 8th, one standard loop from the agar growth of one of the colonies was injected into the peritoneal cavity of a guinea-pig. Next morning the animal, being ill, was killed with chloroform, so as to obviate *post-mortem* changes, and cultures were made from the peritoneal fluid, spleen, and heart's blood. In the spleen and peritoneal fluid a bacillus belonging to the coli group was present in pure culture; the blood, however, contained only a pure culture of *B. typhosus*.

In order to ascertain whether the coliform organism had any connection with the typhoid bacillus a culture was injected into a rabbit. No typhoid agglutinins, however, were produced: in spite of repeated injections the titre of the serum never rose above a dilution of 1—20.

The culture also failed to absorb agglutinins from a known typhoid serum.

Experiment 8.—The culture of *B. typhosus* used in the previous experiment was kept at the laboratory temperature for fourteen days; a standard loop of the agar growth was then injected into

the peritoneal cavity of a guinea-pig. The animal remained perfectly well, and *B. typhosus* in pure culture was recovered from the peritoneal fluid.

It is difficult to explain these irregular results. The guinea-pigs were quite healthy when the experiments were commenced, and *post-mortem* examinations made after the injections revealed no signs of disease. The same amount of culture, 2 milligrammes, was weighed in the standard loop for each experiment, and the guinea-pigs were of approximately the same weight. The only variant was the unknown bodily resistance of each guinea-pig. It will be seen that the *B. typhosus*, which survived in urine for a year, was apparently just as virulent as a strain isolated only nine days after the excretion of the urine. The rapid loss of virulence of the strains after subculture in broth and on agar is worthy of note.

On the Possible Variation of the B. typhosus.

The experiments on the viability of the *B. typhosus* having shown that the vital action of the associated bacteria, and possibly the toxins derived from them, appear to cause a rapid destruction of the typhoid bacillus, it seemed desirable to study the effect of symbiosis with these bacteria and the action of their toxins separately, so as to ascertain whether the *B. typhosus*, under certain conditions, might not change its cultural characters, and so escape recognition.

Symbiosis of B. typhosus with B. fluorescens non-liquefaciens.

A culture of *B. fluorescens non-liquefaciens* was isolated from the urine of "typhoid carrier" S., and planted out in the usual media. The cultural characteristics were as follows :—

Glucose, faintly acid, no gas : mannite, lactose, salicin, dulcite, cane-sugar, unchanged ; neutral red, slight yellow colour ; litmus milk, faintly alkaline ; gelatine, not liquefied, green pigment present ; broth, diffuse growth, green colour present, no indol formation ; morphology, very motile bacillus, Gram negative ; growth at 37° C., good.

A twenty-four hours' broth culture of this bacillus was prepared, and 0·5 cc. of it and 0·5 cc. of a twenty-four hours' broth culture of *B. typhosus* were added to 9 cc. of tap-water. The tube was placed in a dark cupboard of the laboratory.

Dilutions from the mixed growth were then made up to 100,000 millions and plated on MacConkey's bile-salt media. After

forty-eight hours' incubation the plates showed that 95,000 million typhoid bacilli were present in the tube. A week later the contents of the tube had a marked green colour, and 200,000 million colonies of the fluorescent bacillus per cubic centimetre were counted. Further examinations were made at intervals of a week for the next four months. It was soon found impossible to isolate the typhoid bacillus by "direct plating" as the *B. fluorescens* had increased so enormously; but when the dilutions were enriched by growth in MacConkey's bile-salt broth for twenty-four hours and then plated, it was quite easy to separate the two organisms. It was noticed as time went on that the *B. typhosus* diminished in numbers, but at the end of four months it was still present in $\frac{1}{1000000}$ cc. of the original tube, its cultural reactions were quite unchanged, and it was still agglutinated by antityphoid serum.

Result.—The toxins of the *B. fluorescens non-liquefaciens* appear to have no influence in producing variation of the *B. typhosus*.

Symbiosis of B. typhosus and B. coli.

Experiment 1.—In this experiment the symbiosis of *B. typhosus* (R) and a type of *B. coli* isolated from the urine of a typhoid carrier (Bomb. S.) was studied. The cultural characteristics of the *B. coli* were as follows: Glucose, acid and gas; mannite, acid and gas; lactose, acid and gas; salicin, gas, acid slight; dulcite, gas, acid slight; cane sugar, unchanged; litmus milk, acid and clot in seven days; broth, no indol reaction; gelatine, not liquefied; neutral red, gas and yellow colour in seven days; morphology, short bacillus, Gram negative; actively motile. A small particle of a twenty-four hours' growth on agar of each organism was added to 10 cc. of sterilised tap water and the whole thoroughly mixed. Dilutions were then made and plated, and counts showed that 600,000 million typhoid bacilli and 300,000 million colon bacilli were present in each cubic centimetre of the infected water.

Ten days later an examination showed typical colonies of *B. typhosus* and others white and opaque. The latter when fished and planted in the usual media did not ferment any of the sugars in twenty-four hours and the growth on agar was thick and rather opaque. After forty-eight hours there was slight acid in mannite, but no other sugar was fermented even in seven days. The agar growth was planted out again on agar and the resulting growth tested in sugars. Typical reactions of *B. typhosus* were now obtained.

The infected water was plated from time to time and *B.*

typhosus was recovered in gradually decreasing numbers for two months. Examinations at later dates were negative as regards *B. typhosus*, only *B. coli* being isolated.

Experiment 2.—This experiment was carried out in the same manner as the one just described, a different strain of *B. typhosus* (Bombay) being employed. By plating dilutions of the infected water 5,000 million typhoid bacilli and 38,000 million colon bacilli were found in 1 cc.

A week later no signs of typhoid bacilli could be detected. The colon bacilli were still present to the extent of over 1,000 million per cubic centimetre and their cultural characteristics were unchanged.

Experiment 3.—This was a repetition of experiment 2. By plating dilutions, 19,000 million typhoid bacilli and 36,000 million colon bacilli were found in 1 cc. of the inoculated water. A week later more than 1,000 million typhoid bacilli were still present. At the end of two months no typhoid bacilli could be isolated; but on adding 1 cc. of the inoculated water to MacConkey's bile-salt broth and plating on lactose bile-salt litmus agar a few blue colonies were seen. These were found to consist of a bacillus which did not ferment any sugar, and produced an alkaline reaction in milk. At later examinations only *B. coli* was recovered.

Experiment 4.—In this experiment a small particle of a twenty-four hours' agar growth of *B. coli* was added to 2 cc. of urine passed by typhoid carrier I. At the time of the experiment the urine had been kept in a test-tube exposed to light in the laboratory. It was known to contain 10,000,000 typhoid bacilli per cubic centimetre; *B. coli* was also present, but the numbers were only about 1,000 per cubic centimetre. Counts were made frequently, but during the whole time the urine had been kept in the laboratory the *B. coli* had not increased appreciably. After adding the culture of *B. coli* from the agar slope counts showed that the urine contained at least 3,000 million colon bacilli per cubic centimetre.

Three days after the inoculation the urine was again plated in dilutions from $\frac{1}{1000}$ cc. to $\frac{1}{10000000}$ cc. No signs of the *B. typhosus* could be discovered, although the uninoculated urine still showed 10,000,000 typhoid bacilli per cubic centimetre.

The inoculated urine was then enriched by adding 0·5 cc. to 10 cc. of broth. A marked growth occurred after twenty-four hours incubation at 37° C., but on plating the broth no signs of *B. typhosus* could be discovered.

Experiment 5.—This experiment was a control of No. 4; but

instead of a pure culture of *B. coli* a small particle of normal solid fæces was added to the urine. Counts made by plating the inoculated tube showed at least 60,000,000 colon bacilli had been added to the urine. Five days later at least 10,000,000 typhoid bacilli were still present. At the end of a week, however, all the typhoid bacilli had disappeared. Enrichment methods and selective media were tried, but with uniformly negative results.

Remarks.—These experiments show clearly that *B. coli* has an inimical influence on the *B. typhosus*. Experiment 1 indicated that changes were commencing, and in Experiment 2 a variation to a non-fermenting type was apparent.

On the Effect of the Toxins Present in Water after Admixture with Fæces and Urine of Typhoid Carriers.

Nine experiments were made with fæces and twenty-three with urine.

Experiments with Fæces.

In eight of the experiments an attempt was made to extract the toxins present in the dejecta by thoroughly shaking weighed quantities with tap-water until a thin uniform emulsion was produced; this was then filtered, and the filtrate inoculated with a strain of *B. typhosus*. Dejecta from three "carriers" were used, and the toxins were extracted after the fæces had been kept for varying periods at laboratory temperature. In the ninth experiment the rich emulsion of fæces and water was allowed to stand for six days at the laboratory temperature, exposed to light so as to allow toxins to be formed by the bacteria present in the water and fæces. Table B gives the results obtained. It will be seen that the *B. typhosus* was not changed in any way, and its viability apparently depended on the length of time the particular stool had been kept before the extract was made. It is of interest to note that the extract from a stool rich in *B. coli* destroyed the typhoid bacillus more quickly than a corresponding extract from a stool containing comparatively few *B. coli*. The reaction of the filtrate was neutral in each experiment.

Experiments with Urine.

In this series definite quantities of urine were added to water, and the mixture was kept for varying periods in flasks at room temperature and exposed to light. The contents of the flasks were then filtered through sterile Doulton or Pasteur (F) candles; no pressure was employed. The filtrate collected at the end of three

TABLE B.

Name of typhoid carrier	Length of time the stool had been kept at room temperature	Proportion of stool to water	Strain of *B. typhosus* added to the filtrate	Duration of life of *B. typhosus*	Result	Remarks
Gnr. C. ..	3 hours	1 in 500	*B. typhosus*, C.	28 days	*B. typhosus* unchanged	Stool rich in *B. coli* and *B. typhosus* when extracted with water.
Bomb. S. ..	,,	,,	,,	,,	,, ,,	Ditto
Pte. C. ..	3 days	1—1,000	*B. typhosus*, R.	52 days	,, ,,	Stool contained less than 1,000 *B. coli* per grm.; *B. typhosus* still present.
,, ..	5 ,,	1—100	,,	35 ,,	,, ,,	Ditto
,, ..	8 ,,	1—100	,,	31 ,,	,, ,,	Ditto
,, ..	13 ,,	1—100	,,	22 ,,	,, ,,	*B. typhosus* not isolated from the stool.
,, ..	23 ,,	1—100	,,	6 ,,	,, ,,	Ditto
Pte. L. ..	7 ,,	1—100	,,	3 ,,	,, ,,	Stool rich in *B. coli*; *B. typhosus* not isolated at the time the extract was made.
Pte. C. ..	23 ,,	1—100	,,	6 ,,	,, ,,	In this experiment the emulsion of fæces and urine was allowed to stand six days before filtration, so as to allow toxins to be formed by the action of the living bacteria.

hours was tested for sterility and then inoculated with a strain of *B. typhosus*.

Twenty-three experiments were made on these lines, but only four yielded positive results. These will now be considered in some detail.

Experiment I. (33 *in the Register*).—On August 8th, 1909, a specimen of urine from typhoid "carrier" S. was diluted 1 in 10 with tap water, placed in a flask, and exposed to daylight. Eleven days later the mixture of urine and water was filtered through a Pasteur candle (F) without pressure. The filtrate collected after three hours was plated on large bile-salt neutral red lactose agar plates, 0·5 cc. of the filtrate being carefully spread over the surface of the solid agar in the plates. The plates remained quite sterile after prolonged incubation at 37° C. The filtrate was then inoculated with a small particle of a forty-eight-hours' agar growth of *B. typhosus*, isolated from the stool of "carrier" C. After thoroughly mixing the agar growth with the filtrate, dilutions were made and plated; the counts showed that the inoculated filtrate contained 480,000,000 typhoid bacilli per cubic centimetre.

On August 25th, 1 cc. of the inoculated filtrate was diluted up to 1,000,000,000, and plated on bile-salt neutral red lactose agar. In the plate containing $\frac{1}{10000}$ cc. six colonies were seen; two of these were fished and planted out in the usual media. The following results were obtained:—

	Glucose	Mannite	Lactose	Salicin	Dulcite	Cane sugar	Litmus milk	Broth	Gelatine	Neutral red	Motility	Agglutination with typhoid serum
Col. 1 ..	0	Acid	0	0	0	0	Acid, then alkaline	No indol	NL	0	+	0
Col. 2 ..	0	0	0	0	0	0	Unchanged, then alkaline	No indol	NL	0	+	0

In the plate containing $\frac{1}{1000}$ cc. there were numerous colonies like those of *B. typhosus*; two were fished and planted out in the above media. The first colony gave all the reactions of *B. typhosus;* the second colony appeared to be identical with colony 2 in the $\frac{1}{10000}$ cc. plate.

On August 28th the inoculated filtrate was again diluted and plated; colonies were fished and planted out as before; none of these fermented any of the sugars, and they appeared to be identical with colony 2, isolated on the previous occasion.

The inoculated filtrate was again examined during September, October, and November, 1909; no signs of the *B. typhosus* were

ever discovered; all the bacteria present gave the reactions of colony 2.

Experiment II. (35 *A in the Register*). — A sample of urine from a typhoid carrier S., passed on September 24th, 1909, and containing 3,890,000 typhoid bacilli when received next day, was diluted 1 in 10 with tap water, and placed in a cupboard of the laboratory, protected from light. On October 8th the mixture of urine and water, which was now turbid and yellow in colour, was filtered through a sterilized Pasteur candle (F), at ordinary atmospheric pressure. At the end of three hours the filtrate obtained was placed in two sterile test-tubes, and 2 cc. of it was added to sterile broth; after seventy-two hours incubation at 37° C. the broth tube remained quite sterile. One of the tubes containing the filtrate was then inoculated with a small loopful of a twenty-four-hours' agar culture of *B. typhosus* (R), the stock typhoid culture employed in the manufacture of vaccine at the Royal Army Medical College. The inoculated tube and the uninoculated tube, which served as a control, were placed in a dark cupboard. On October 14th and 20th a portion of the contents of the inoculated tube was diluted and plated on neutral red bile-salt lactose agar; 338,000,000 typical typhoid bacilli per cubic centimetre were isolated. On October 25th the control tube was plated in the same manner; no growth occurred in any of the plates. On November 11th dilutions of the inoculated tube were again plated, in the plate containing $\frac{1}{1000000}$ cc. forty-two colonies appeared; one of these, labelled 35 A, colony 1, gave all the reactions of the typhoid bacillus (R), but twelve of the remaining colonies did not ferment glucose; one of these, labelled 35 A, colony 2, was then planted out in the usual media, when the following results were obtained:—

	Glucose	Mannite	Lactose	Salicin	Dulcite	Cane sugar	Litmus milk	Broth	Gelatine	Neutral red	Motility	Agglutination with anti-typhoid serum
35A, Col. 2..	0	0	0	0	0	0	Acid	No indol	Not liquefied	0	+	0
35A, Col. 1..	A No G	A No G	0	0	A No G	0	Acid	No indol	Not liquefied	0	+	+ (1—1,000)

On November 11th the contents of both tubes were again plated. The plates made from the contents of the inoculated tube showed colonies, all of which gave the same reactions as 35 A, colony 2, just described, except that the litmus milk, which was first rendered acid, became distinctly alkaline at the end of seven days' incubation at 37° C.; no signs of the *B. typhosus* could

be found in the plates. The contents of the control tube showed no growth after the plates had been incubated for four days.

On January 1st, 1910, a large loopful of the inoculated filtrate was plated on glucose bile-salt litmus agar and 1 cc. of the control filtrate was added to broth. The control after seven days' incubation at 27° C. showed no growth, but in forty-eight hours blue colonies appeared in the glucose plates. These colonies when tested in the usual media again failed to ferment any sugar and produced an alkaline reaction in milk.

On February 3rd and March 2nd, 1910, similar results were obtained. On March 24th a loopful of the inoculated filtrate was again plated, but no growth occurred, showing that the bacteria were diminishing in numbers. Four cubic centimetres of the inoculated filtrate were then added to broth, which was incubated at 37° C. A growth occurred in forty-eight hours and a loopful of this was plated on MacConkey's medium. In the plates large and small colonies appeared; the large colonies consisted of a Gram negative motile bacillus which gave the same reaction as 35 A; the small colonies were made up of a rather large Gram positive coccus.

The latter organism when tested gave the following reactions: Glucose, acid, no gas; mannite, acid, no gas; lactose, acid, no gas; salicin, acid, no gas; dulcite, unchanged; cane sugar, acid, no gas; broth, diffuse growth, no indol; litmus milk, acid and clot; gelatine, not liquefied; neutral red (anaerobic condition), yellow. This streptococcus has a close resemblance to the *Streptococcus fæcalis*. It is possible, of course, that the streptococcus might have been a contamination introduced from without, in view of the length of time the inoculated tube had been under observation and the frequency with which it had been opened. Experiments to be recorded later, however, seem to show that the streptococcus might have been produced from the bacillus present in the contents of the inoculated tube.

Experiment III. (37 *in the Register*). A specimen of urine passed by "carrier" I. on September 24th, 1909, and containing more than a million typhoid bacilli when received next day, was diluted 1 in 10 with tap water and poured into a flask, which was then plugged with cotton wool and placed in a cupboard of the laboratory. On October 8th, the mixture of urine and water was filtered through a sterilised Doulton candle at atmospheric pressure. The filtrate obtained at the end of three hours was placed in two sterile test-tubes and 2 cc. of it was added to sterile broth. After seventy-two hours' incubation at 37° C., the broth tube showed no signs of

growth. One of the tubes containing the filtrate was now inoculated with a small loopful of a twenty-four-hours' agar culture of *B. typhosus* (R). The inoculated tube and the second tube, which served as a control, were then placed in a dark cupboard.

On October 14th, the contents of the inoculated tube were plated and typical typhoid colonies, giving the same reactions as *B. typhosus* (R), were obtained.

On October 20th, an examination showed only typical typhoid bacilli to be present in the inoculated tube. On November 4th, typical typhoid bacilli were once more isolated from the inoculated tube.

On November 10th the contents of the inoculated tube and the control tube were plated. The control tube proved to be quite sterile, but in the inoculated tube no signs of the *B. typhosus* could now be discovered, it appeared to be replaced by a bacillus giving the same cultural reactions as 35 A in experiment II.

Experiment IV.—In this experiment the urine of "carrier" S. was mixed with an equal quantity of water in a flask, and exposed to light in the laboratory for four months. The mixture was then filtered through a sterile Doulton candle without pressure; only 12 cc. of filtrate was obtained at the end of two hours, of this 2 cc. was added to broth and incubated at 37° C. during the whole time the experiment lasted; no growth occurred in the broth. The 10 cc. of filtrate remaining was inoculated with a small particle of a twenty-four hours' agar growth of *B. typhosus* (R). A week later $\frac{1}{10}$ cc. of the inoculated filtrate was plated, but no growths occurred in the plates. Three cubic centimetres of the inoculated filtrate were then added to broth and incubated at 37° C. After three days' incubation the broth showed a growth, which was plated on glucose bile-salt litmus agar; after twenty-four hours at 37° C. the plates showed only blue colonies, consisting of bacilli, which, when subcultured, did not ferment any sugar, and produced a faintly acid reaction in milk. The microbe appeared to be identical with 35 A when that organism was first isolated.

The details of the remaining nineteen experiments are summarised in Table C. It will be seen that the reaction of the filtrates varied considerably; in those which became markedly alkaline the duration of life of the *B. typhosus* was short. That the viability of *B. typhosus* does not depend entirely on the reaction of the medium is evident from the results of Experiments 21 and 22.

TABLE C.

No. of experiment	Source of urine	Dilution with water	No. of days before filtration	Strain of *B. typhosus* planted in the filtrate	No. of days *B. typhosus* survived	Result	Remarks
5	Recent case of enteric fever, A.	1—100	5	*B. typhosus* (R)	4 months	*B. typhosus* unchanged	Reaction of filtrate not tested
6	do.	1—10	5	,, (Bombay)	3 ,,	do.	Reaction of filtrate neutral
7	do.	1—10	5	,, (A)	4½ ,,	do.	do.
8	do.	1—10	9	,, (A)	5 ,,	do.	do.
9	do.	1—10	9	,, (R)	4½ ,,	do.	do.
10	do.	1—10	13	,, (R)	4½ ,,	do.	do.
11	do.	1—10	13	,, (A)	5 ,,	do.	do.
12	do.	1—100	13	,, (R)	6 weeks	do.	Control not sterile after prolonged incubation. Experiment abandoned
13	do.	1—100	13	,, (A)	2½ months	do.	Reaction alkaline, —6
14	Carrier I. ..	1—1	26	,, (R)	8 days	do.	do. —22
15	,, S. ..	1—10	7	,, (S)	11 ,,	do.	do. —30
16	,, S. ..	1—10	7	,, (I)	5 ,,	do.	Filtrate of 15, neutralised, refiltered, and again inoculated
17	,, I. ..	1—10	42	,, (R)	5 ,,	do.	Filtrate alkaline, —30
18	,, S. ..	1—100	7	,, (R)	2½ months	do.	Reaction not tested
19	,, S. ..	1—10	18	,, (R)	15 days	do.	Reaction alkaline, —30
20	,, S. ..	1—10	18	,, (I)	15 ,,	do.	Filtrate of 19 neutralised and refiltered
21	Recent case of enteric fever, P.	1—10	21	,, (E)	2 months	do.	Reaction neutral
22	do. S.	1—10	36	,, (E)	21 days	do.	do.
23	Carrier S.	1—10	14	,, (R)	5 months	do.	Reaction not tested

Further Tests to see if the Bacillus, 35 A, Described in Experiment II. is Able to Absorb and Produce Agglutinins Specific for the B. typhosus.

The culture was planted out on three agar slopes, and the resulting growths were then used to absorb the agglutinins from a known anti-typhoid serum. After absorption the serum was tested with a laboratory culture of *B. typhosus* (strain Bombay).

Another portion of the same serum was absorbed with a culture of the *B. fluorescens non-liquefaciens* (isolated from carrier S.'s urine) and then tested with *B. typhosus* (strain Bombay). The following results were obtained :—

	Dilutions of serum untreated		
	1—100	1—500	1—1000
Tested with *B. typhosus* (Bombay)	+	+	+
	Dilutions of serum absorbed with *B. typhosus* (Bombay)		
,, ,, ,, ...	0	0	0
	Dilutions of serum absorbed with culture 35 A		
,, ,, ,, ...	+	+	0
	Dilutions of serum absorbed with *B. fluorescens*		
,, ,, ,, ...	+	+	+

As the culture 35 A appeared to have a slight power of absorbing agglutinins from antityphoid serum, it was decided to inject the culture into a rabbit to see if agglutinins specific for the *B. typhosus* would be produced. The following results were obtained :—

	Dilutions of rabbit's serum			
	1–20	1—40	1—80	1—160
Tested with Bacillus 35 A	+	+	±	0
B. typhosus (R)	+	±	±	0

In spite of repeated injections the titre of the serum remained as shown in the table. The agglutinins were completely removed by absorbing the serum with either *B. typhosus* or culture 35 A.

These results being indecisive, it was then determined to try the effect of intraperitoneal passage through a series of guinea-pigs. It was thought that if the bacillus (culture 33) isolated in Experiment I., and the bacillus (35 A) obtained in Experiment II., were derived from the *B. typhosus*, passage through a warm-blooded animal might cause these bacteria to revert to the original type.

Passage of Culture 33 through a Series of Guinea-pigs.

A twenty-four hours' agar growth of the culture was emulsified in normal salt solution, and, approximately, one-tenth of the slope was injected into the peritoneal cavity of a guinea-pig, the fluid withdrawn after an interval of three hours was subcultured on agar,

and $\frac{1}{10}$ of the growth then injected into another guinea-pig. The peritoneal fluid was removed after eighteen hours, planted on an agar slope, and the resulting growth injected into a third guinea-pig. After three passages the culture was unchanged except in one experiment, in which the fluid was removed from the same guinea-pig after six hours and again after twelve hours. The fluid removed after six hours contained a short Gram negative bacillus, like *B. coli*, which produced acid and gas in glucose, lactose, mannite, cane sugar, dulcite, and salicin; it also clotted milk, produced indol in broth, and a yellow colour in neutral red. The fluid removed after twelve hours, however, contained a bacillus which gave the same reactions as the bacillus originally injected into the peritoneal cavity.

There appear to be two possible explanations for this result: (*a*) The *B. coli* was derived from the intestine of the guinea-pig, or (*b*) the culture 33 was changing its cultural characters, but was not yet stable and reverted to its original shape after twelve hours in the peritoneal cavity. Experiments to be recorded later suggest that the latter is the more probable explanation.

Passage of Culture 35 A through a Series of Guinea-pigs.

The culture was left in the peritoneal cavity of each guinea-pig of the series for a period gradually increasing from six to eighteen hours. After six passages the culture was quite unchanged, but the fluid removed after the eighth passage, when planted in broth, gave a growth consisting of cocci, or very short bacilli in pairs and short chains. The broth was plated on agar; two kinds of colonies appeared: (1) Medium sized, made up of bacilli, like the original 35 A; and (2) smaller colonies, consisting entirely of cocci in short chains. Colony (1) was fished and planted in broth; a growth of cocci appeared. This was then planted on agar, when only bacilli were seen. The agar slope was then tested in sugars, &c., with the following results: Glucose, acid, and gas; lactose, acid, no gas; mannite, acid, no gas; cane sugar, acid, no gas; dulcite, unchanged; litmus milk, acid and clot; neutral red, gas; gelatine, not liquefied.

As the morphology of this organism was found quite unstable, Gram negative bacilli or cocci appearing in the various media employed, it was determined to repeat the intraperitoneal passages, commencing this new series with a fresh culture isolated from the inoculated filtrate on January 1st, 1910. Before injection this culture was tested in sugars, &c., and found to give the original

reactions of 35 A. From the first to the fourth passage the culture was left in the peritoneal cavity of each guinea-pig for six hours, from the fifth to the eighth passage for eighteen hours, and from the ninth to the seventeenth passage for twenty-four hours. Up to this passage the culture remained absolutely unchanged, but after the eighteenth passage it gave the following reactions: Glucose, acid and gas; mannite, acid and gas; lactose acid, no gas; salicin, acid, no gas; dulcite, unchanged; cane sugar, unchanged. This culture was at once injected into another guinea-pig, making the nineteenth passage. *The growth obtained from the peritoneal cavity of this guinea-pig did not ferment any sugar, and gave the same cultural reactions as the culture at the commencement of the series of passages.* This result is extremely interesting as it seems to indicate clearly that as the culture, which after the eighteenth passage fermented certain sugars with the production of acid and gas, reverted to the original type, it must have been derived from the original culture, and was not a contamination from the peritoneal cavity. A subculture from the agar growth obtained at the eighteenth passage was planted from agar to agar and the third subculture obtained, which still fermented glucose, lactose, mannite, and salicin, was again passed through the peritoneal cavity of a fresh series of guinea-pigs. After six passages the same sugars were still fermented, but the gas formation was increased; litmus milk was also clotted with an acid reaction, and a yellow colour was produced in neutral red; there was, however, no indol formation in broth. Two further passages were made, but the culture still gave identical reactions. The culture, which reverted to the original type on the nineteenth passage was subjected to six more passages, but no change in the cultural reactions occurred, the bacillus still did not ferment any sugar, and caused an alkaline reaction in milk. At the next passage, however, a pure growth of a Gram negative bacillus was obtained, which produced slight acid in glucose, lactose, salicin, and cane sugar. On planting this bacillus in broth, chains of Gram positive cocci appeared mixed with bacilli, and on plating this broth culture two kinds of colonies were obtained—*i.e.*, (1) Medium-sized white colonies made up of Gram negative bacilli which did not ferment any sugar and produced an alkaline reaction in milk, and (2) small colonies made up of Gram positive cocci which produced acid, but no gas, in glucose, mannite, lactose, cane sugar, and salicin, and clotted milk. The Gram positive coccus was passed through three guinea-pigs, but maintained its characters, no signs of bacilli being seen.

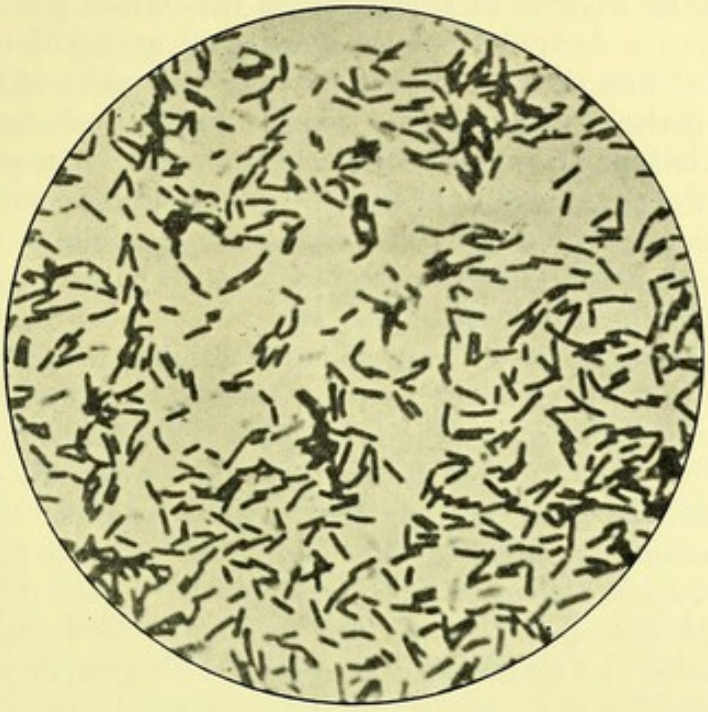

FIG. 1.—Growth on agar after incubation for twenty-four hours at 37° C. × 1000.

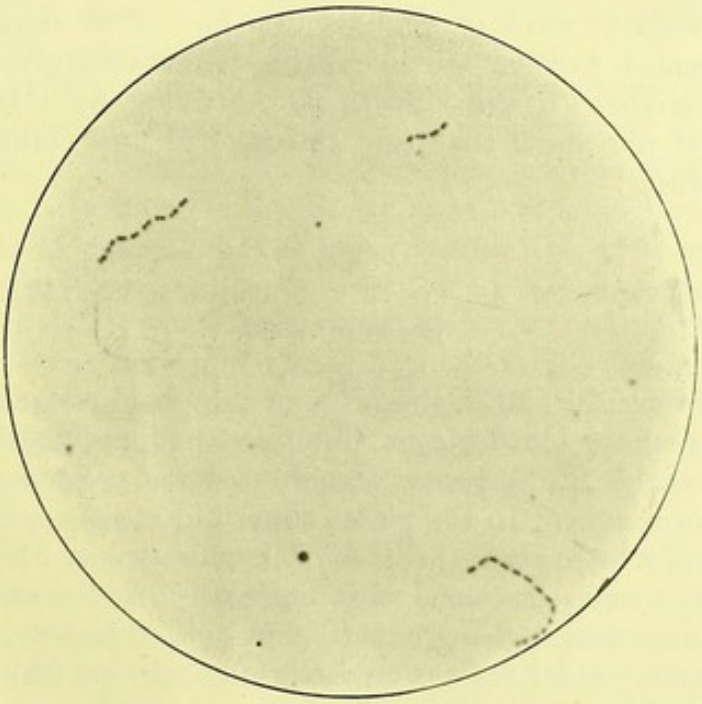

FIG. 2.—Growth on agar planted in broth and incubated for twenty-four hours at 37° C. × 1000.

It would be natural to suppose that this Gram positive coccus was an impurity derived from the peritoneal cavity of the guinea-pig. Against this view is the fact that the agar slope of the growth from the peritoneal cavity was kept for a week and stained frequently, but no Gram positive cocci appeared. On planting the growth on the agar slope in broth, however, definite chains of Gram positive cocci appeared in twenty-four hours (see figs. 1 and 2). It should also be noted that the streptococcus is apparently identical with that obtained in Experiment II.

The breaking up of a Gram negative bacillus into a Gram positive coccus in both series of passages seemed so extraordinary that it was determined to repeat the first series of passages, commencing, however, with the culture in this series which was quite unchanged in its characters after six passages. After passing this culture through another guinea-pig, this being the seventh passage, the bacillus was again recovered quite unchanged. At the next passage, however, the agar growth from the peritoneal fluid showed Gram negative bacilli and Gram positive cocci. This growth was planted in broth, next day the same bacilli and cocci were seen. The broth was plated and colonies, some consisting apparently of bacilli and others of cocci, appeared; when fished and planted out in broth, each of the colonies again produced Gram positive cocci mixed with bacilli. Owing to want of animals this line of investigation could not be pursued further. The results, while strongly pointing to a definite change in the culture 35 A, Col 2 by intraperitoneal passage, did not show that this culture had any connection with the *B. typhosus*.

Further Evidence as to the Possible Variation of the B. typhosus.

On October 12th, 1910, the urine of a typhoid convalescent case, T. S., from whose blood Major Cummins had previously isolated a pure culture of *B. typhosus*, was diluted and plated on bile-salt glucose litmus agar. In the plates containing $\frac{1}{1000000}$ cc. a bacillus was present which after three days' incubation at 37° C. gave the characteristic sugar reactions of *B. typhosus*. At the end of a week, however, there was slight production of acid in lactose, salicin, and dulcite, marked indol formation in broth, and neutral red showed a slight yellow colour; moreover, the bacillus was only feebly motile, and was not agglutinated by anti-typhoid serum. Obviously

at this time the urine of the patient contained a micro-organism intermediate between *B. typhosus* and *B. coli*.

The culture, labelled S. bacillus, was then injected into a rabbit to see if agglutinins specific for the *B. typhosus* would be produced. Ten days later the serum of the rabbit was tested with a laboratory strain of *B. typhosus* and with the homologous bacillus; the following results were obtained:—

	Dilutions of serum					
	1—10	1—50	1—100	1—200	1—500	1—1,000
Homologous bacillus ..	±	0	0	0	0	0
B. typhosus, E	+	+	+	+	±	0

The rabbit was then given a second injection and a week later the serum was again tested for agglutinins and opsonins. It was then found that the titre of the typhoid agglutinins had fallen and the laboratory strain of the typhoid bacillus was not completely agglutinated by the serum diluted only $\frac{1}{100}$. Major Cummins, who kindly tested the serum for typhoid opsonins, reported that the rabbit appeared to be suffering from a negative phase, the phagocytes ingesting only about half as many bacteria opsonised with the serum as compared with the control specimens made with normal serum. A week later, however, the agglutinins for the *B. typhosus* had again increased and the results given in the previous table were obtained once more. Moreover, at this time a marked opsonic reaction for *B. typhosus* was given by the serum.

The S. bacillus was next tested as to its power of absorbing agglutinins from a known typhoid serum which had a titre 1,000 for a laboratory strain of the *B. typhosus*. It was found that all the agglutinins specific for the *B. typhosus* were removed.

It was then determined to apply the test of deviation of complement. Captain L. W. Harrison, who has had considerable experience of this test, carried out the necessary manipulations, and reported that the serum of the S. bacillus rabbit deviated complement in the same manner as a known anti-typhoid serum. The effect of intraperitoneal passage through guinea-pigs was next tried, controlling each passage by subculturing the original culture of S. bacillus from agar to agar.

After four passages of the bacillus the power of fermenting lactose appeared to be lost and the micro-organism only differed from a typhoid bacillus in that it was feebly motile and produced a trace of acid in salicin. It was thought that by continuing the passages a true *B. typhosus* might be obtained. After four more passages, however, there was a complete reversion to the original

type and the cultural characters were exactly the same as those of the strain cultivated from agar to agar.

A second specimen of urine passed by the typhoid convalescent, T.S., on November 17th, 1910, was diluted and plated on November 18th, to see if the S. bacillus just described was being excreted continuously. No signs of it were detected, but 1 cc. of the urine now contained one million bacilli giving all the cultural reactions of *B. typhosus*. This bacillus was motile, readily agglutinated by antityphoid serum in a dilution of 1 in 1,000 and also absorbed all the agglutinins from a known typhoid serum.

Conclusions.

I. *The Viability of the B. typhosus.*

The experiments seem to indicate that the duration of life of the *B. typhosus*, as at present recognised, is very short under natural conditions; it is unlikely that the artificial conditions in a sterile test-tube, under which the typhoid bacillus survived for over a year, will find a parallel in nature. Whenever typhoid urine and fæces gain access to wells, springs, or cesspools, it seems probable that the action of the associated bacteria will cause the typhoid bacillus to disappear in a few days.

Soil bacteria appear to have a similar destructive power. Also when fæcal material is lightly covered with earth, or exposed to the atmosphere, the colon bacilli present exercise a marked inimical influence on the typhoid bacilli. If the dejecta contain many *B. coli*, millions of typhoid bacilli disappear in two or three days, and even if the typhoid bacilli are a thousand times more numerous than the *B. coli*, a similar result follows in about a week.

II. *The Possible Variation of the B. typhosus.*

(1) Symbiosis of the *B. fluorescens non-liquefaciens*, present in the urine of typhoid carriers, and the *B. typhosus* does not cause any change in the cultural character of the latter microbe.

(2) Symbiosis of *B. coli* and *B. typhosus* appears to cause a rapid destruction of the typhoid bacillus; in two experiments there was evidence of a change in the typhoid bacillus, but many more experiments must be undertaken before a definite statement can be made on this head. Possibly, if a smaller dose of *B. coli* had been used more definite results would have been obtained.

(3) The toxins extracted by water from the fæces of typhoid

carriers do not appear to produce any changes in the cultural characters of the *B. typhosus*.

(4) The toxins formed in a mixture of one part of urine from a typhoid carrier and nine parts of tap water after varying periods at the laboratory temperature, in four instances caused the typhoid bacillus to change into a bacillus having a close resemblance to the *B. fæcalis alkaligenes*. Intraperitoneal passage through a series of guinea-pigs did not again convert this bacillus into the *B. typhosus*, but a bacillus having many of the characters of *B. coli* appeared. When introducing a foreign microbe into the peritoneal cavity of a guinea-pig it is always possible that the injection may cause a change in the coats of the intestines, and so lead to a passage of *B. coli* from the intestine into the peritoneal cavity. This might be the explanation of the results obtained in the intraperitoneal passages made in connection with experiments I. and II., but that a real variation of the bacillus into a coli type occurred seems probable from the following facts:—

(*a*) In the intraperitoneal passage made with the bacillus 33, after three passages the *B. coli* type was recovered after six hours and the original type after twelve hours, from the *same* guinea-pig. If the *B. coli* type recovered after six hours had been an exudation from the bowel, it should have been present after twelve hours in even greater numbers. It might, however, be argued that the *B. coli* exuded had been destroyed by phagocytes after twelve hours, and the bacillus then recovered was derived from those originally injected which had proved more resistant to the action of the phagocytes.

(*b*) In the experiments with bacillus 35 A the same change into a coli type occurred in three series of passages. In one of the series, on injecting the coli type recovered after eighteen passages into another guinea-pig, a reversion to the original type occurred.

(*c*) The guinea-pigs were killed with chloroform and a careful *post-mortem* examination revealed no signs of peritonitis.

(5) A mutation, *i.e.*, a complete change of form as well as of the cultural characters, of the bacillus 35 A through a coli type into a streptococcus having the cultural characters of *S. fæcalis* occurred as a result of intraperitoneal passage. The objections mentioned under (4) might be advanced against this change, but the following points in favour of a mutation appear to have considerable weight:—

(*a*) The mutation from the coli type to the Gram positive streptococcus was gradual, at first the cocci obtained did not retain the Gram stain.

(*b*) The coccus type at first was very unstable, a change to a bacillary type readily occurring on changing the nutrient medium.

(*c*) The streptococcus obtained in each series had the *same* cultural characters.

(6) The isolation of the S. bacillus from the urine of a patient who was known to have suffered from a definite attack of enteric fever appears to be a strong proof of the possible variation of the *B. typhosus*. The S. bacillus had cultural characters intermediate between *B. typhosus* and *B. coli*, yet it produced in an animal agglutinins specific for the typhoid bacillus, removed agglutinins from a known typhoid serum, formed opsonins for the typhoid bacillus, and finally produced a serum which deviated complement in the same manner as a known typhoid serum.

PARATYPHOID FEVER IN INDIA.

By Major H. W. GRATTAN and Captain J. L. WOOD,
Royal Army Medical Corps.

Recent experiences, as the outcome of the application of modern laboratory methods in the diagnosis of the pyrexias, have emphasized the extensive prevalence of paratyphoid infection among European troops serving in India. The question of paratyphoid fever has received, and is receiving, considerable attention in Europe. Judging by some recent papers on the subject,[1] we infer that European writers are more or less unanimous in applying the term paratyphoid fever to the infection caused by the *Bacillus paratyphosus* B, and that they regard this micro-organism as the most common representative of the paratyphoid bacilli to be met with in, so-called, paratyphoid fever as observed at home and in Europe generally. We believe that it is not generally recognized that paratyphoid fever is widespread in India, and, further, still less appreciated that the causal micro-organism of the paratyphoid fever of India is not the B variety, but the *B. paratyphosus* A. The records of cases due to the B variety in India, where the diagnosis was based on the isolation of this micro-organism from the blood, are very few and not free from criticism. The Enteric Fever Commission in India, 1906-8, described two cases of fever due to the *B. paratyphosus* B; in one case the bacillus was isolated from the urine, and the blood serum of the other case in a dilution of 1 in 1,500 agglutinated the bacillus. The present paper contains the results of our own experiences and is a plea for a wider view, if not an actually different view, as to what is the cause of the clinical entity we call paratyphoid fever. Before we discuss the definition, symptomatology and epidemiology of paratyphoid fever in India, it may be useful to refer briefly to some previous literature on the same subject.

Paratyphoid fever has been recognized in India only since the causal micro-organisms were isolated from patients by the members of the Enteric Fever Commission of 1906-8, when the *B. paratyphosus* A was isolated from four patients.[2] In the cold

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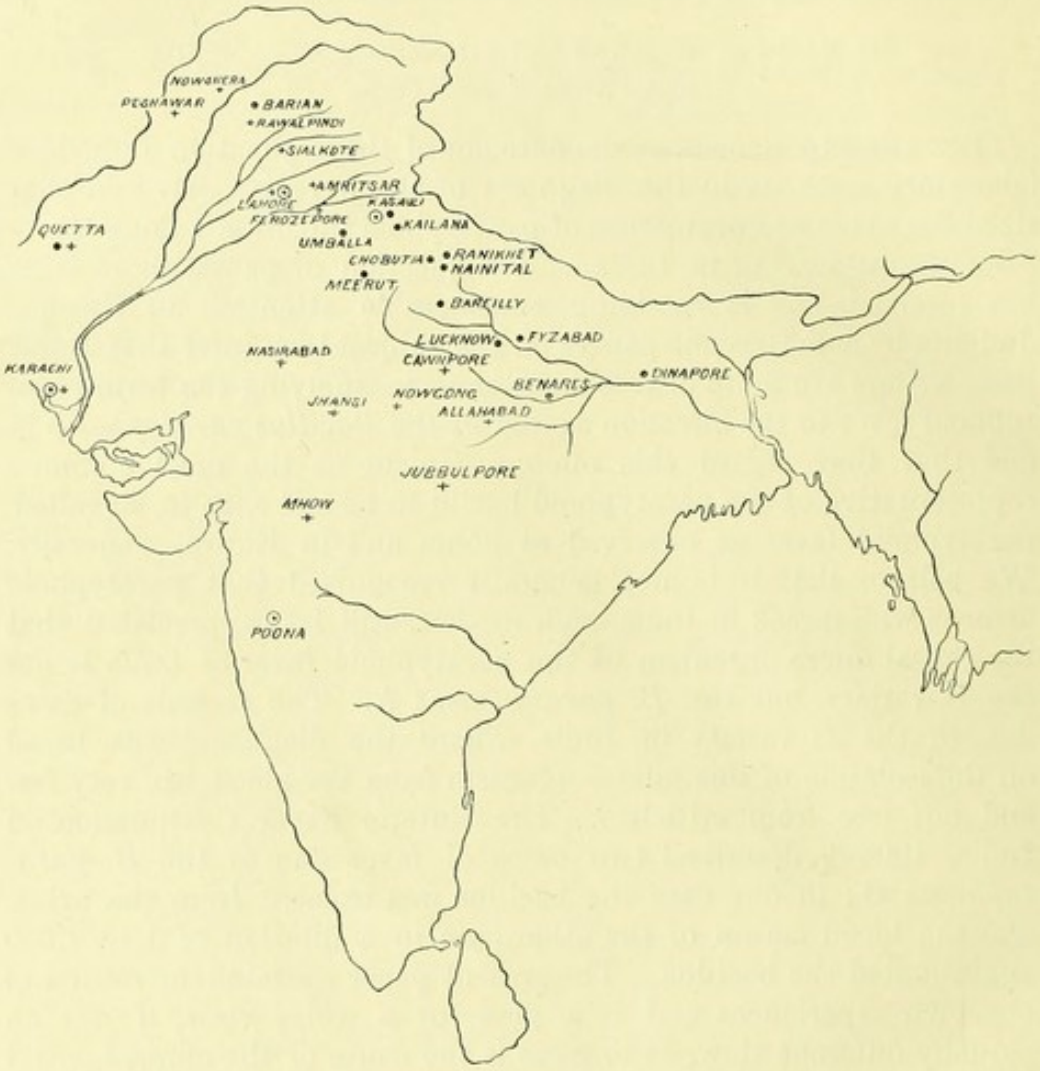
[1] See papers by Bainbridge and Dudfield, Bainbridge and O'Brien; also by Trommsdorff, Rajchman and Porter in *Journal of Hygiene*, vol. ix, No. 1, March, 1911.

[2] "Scientific Memoirs," issued by Government of India, N. S., No. 32, 1908.

Paratyphoid Fever in India



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The spot • indicates stations where men have contracted paratyphoid fever and from whom the *Bacillus paratyphosus* A was recovered at the time or during convalescence, and was identified in this laboratory.

The spot ⊙ indicates that this bacillus was recovered from the blood of patients, and was identified in other laboratories.

The cross + indicates stations where men have contracted a fever which produced in their blood agglutinins for *B. paratyphosus* A. The charts and case sheets of these patients were in keeping with the diagnosis of paratyphoid fever suggested by the Gruber-Widal reaction.

weather of 1908-9, Captain Morrison, I.M.S., isolated the *B. paratyphosus* A from the blood of two cases in Lucknow. One of us also cultivated the same micro-organism from the blood of two patients at Bareilly about the same time. In 1909, Major D. Harvey, R.A.M.C., described ten cases of paratyphoid fever due to the A variety of the bacillus, which had been isolated from the blood or excreta of these patients at Naini Tal.[3] Further, in 1910, Major D. Harvey and one of us described a small epidemic of eight cases of paratyphoid A infection at Naini Tal, the causal organism having been isolated from the blood of seven of these patients.[4] Lately, the same writers investigated a similar outbreak at Benares caused by an acute carrier.[5]

Definition.

In this paper, where writing of paratyphoid fever, we refer entirely to fever caused by the *B. paratyphosus* A, unless the contrary is stated, and, in attempting to define paratyphoid fever, we would describe it as an acute septicæmic fever due to the presence in the blood stream of the *B. paratyphosus* A. It is a fever which varies clinically within wide limits, from the ambulant case on the one hand, which does not come under the notice of the medical officer, to the severe case on the other hand, which is diagnosed as enteric fever clinically, and from which disease it can only be differentiated by bacteriological methods.

Symptomatology.

The incubation period, which is marked by headache and general malaise, is about the same as in enterica; but as the period varies in this disease, chiefly, we imagine, according to the dose and virulence of the bacillus coupled with the resisting power of the patient, so it varies in paratyphoid fever. We have recovered the bacillus from the blood of two patients who undoubtedly contracted the fever within ten or fourteen days of landing in India. We are of opinion that the average incubation period is fifteen days.

The onset is gradual—the patient complains of severe frontal headache and pains in the back and limbs; the attack might easily be mistaken for the onset of influenza, or even rheumatic fever.

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[3] Journal of the Royal Army Medical Corps, April, 1910.
[4] *Ibid*, January, 1911.
[5] *Ibid.*, August, 1911.

In about half the cases there are symptoms of bronchitis and sore throat. The conjunctivæ are suffused and the tongue is moist and furred. When the temperature is recorded from the commencement of the illness the characteristic "staircase" rise is seen and it reaches its maximum by the fifth or seventh day. The fever does not rise as high as in enterica, 102°·5 to 103°·5 being the average highest points reached. The fall is by lysis and reaches normal about the ninth to the fourteenth day. The pulse differs from that of enterica, it is more rapid and more in keeping with the temperature. A pulse of 112 per minute is quite a common feature, in contrast to the slower pulse of enterica, and is not so much an indication of a severe attack of the fever as would be the case in the more serious disease.

The respirations are increased in proportion to the degree of lung complications present. The abdomen is slightly distended and the abdominal reflex is absent, some enlargement of the spleen and liver may be noticeable. Constipation is the rule. We have observed a variety of rashes (in addition to "rose spots") of a morbilliform, erythematous and purpuric character; a rash is not so common as in enterica. Epistaxis occasionally occurs.

Patients are not nearly so dull and heavy as in enterica, they are much brighter, and take more interest in their surroundings. The above is a description of an ordinary case of the fever (*vide* Chart 4). We recognize two other distinct types: (2) the mild, and (3) the severe.

In a mild attack, the patient may report sick with "a touch of fever," which may easily be mistaken for malaria. Chart 1 illustrates such a case, which would have been overlooked if the *B. paratyphosus* A had not been isolated from the blood. This patient was not at all ill in the ordinary sense of the term.

The severe type is clinically indistinguishable from an attack of enterica. The fever lasts from twenty-one to thirty-six days, and may be accompanied with all the clinical manifestations of ordinary enteric fever, and occasionally with those of a severe attack of that fever (*vide* Chart 2). Toxæmia, however, is not so marked as in the more serious disease, and convalescence is more rapid.

Complications.

The very mild attack may be followed by a relapse, and in our experience the relapse is more common than in enterica, probably on account of the smaller amount of care taken with regard to food, &c., both by the patient and attendants, owing to the mild nature

of the disease and the rapid convalescence. In many cases the relapse begins with a sudden rise of temperature, tenderness over the gall-bladder, and a "dysenteric" attack. The abdominal reflex is diminished, or lost during the relapse.

Phlebitis and "Enteric Leg" are almost as common as in enterica. Perforation occurred once in our experience (the *B. paratyphosus* A was recovered from the gall-bladder).

Cholecystitis is not uncommon and manifests itself by acute pain over the gall-bladder and a sharp rise of temperature. Chart 5 illustrates the type of fever associated with this condition.

Anæmia.—An ordinary attack of the fever may produce a degree of anæmia (as judged by the appearance of the patient) quite out of proportion to the severity of the disease.

Pneumonia and Bronchitis are common, but are in reality part of the disease and hardly complications.

Laryngitis.—We have seen one case of laryngitis with partial loss of voice. We have not observed peripheral neuritis or arthritis.

Morbid Anatomy.

We only know of three cases which died from paratyphoid fever and in which the diagnosis was made certain by blood culture. In only one of these cases was a post-mortem examination made. In this instance the man died from perforation of the only ulcer which could be found at the autopsy. The Peyer's patches were not obviously enlarged, but the lower part of the ileum showed signs of inflammation, the spleen weighed 6 oz., the wall of the gall bladder was thickened, and on microscopical examination its lining membrane was found to be the seat of a small round cell infiltration. The bile contained a pure culture of *B. paratyphosus* A.

Bacteriology.

The isolation of the causal organism from the blood stream is a comparatively easy matter during the first four or five days of the pyrexia. The chances of a successful blood culture are greatly diminished by the eighth day, even when the usual 5 cc. of blood is withdrawn—in marked contrast to the case in enterica. We prefer to use sterilized ox bile as the culture medium, and after twenty-four hours incubation sub-cultures from the mixture of blood and bile are made on to the original Conradi-Drigalski medium. All the members of the typho-colon group grow readily on this medium and are not inhibited, as is the case on the more modern selective media. After a further twenty-four hours incubation the

colonies are tested with sera specific for *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B. The *B. paratyphosus* A is indistinguishable in appearance from *B. typhosus* on Conradi-Drigalski plates.

Characters.—A non-Gram staining short motile bacillus, which is agglutinated at once by specific serum. In media composed of 1 per cent peptone and 0·5 per cent sodium chloride in water to which 1 per cent of the following sugars and alcohols have been added, the bacillus gives the following reactions in twenty-four to forty-eight hours, which do not alter up to ten days, although some re-absorption of gas may take place and fluorescence disappear.

Glucose	Lactose	Mannite	Cane sugar	Dulcite
Acid and gas	No change	Acid and gas	No change	Acid and gas

The amount of gas formed in glucose is small, the Durham's tube may show none, or at the most be a quarter full of gas. There may be no change in dulcite for two or three days when abundant gas is formed. The amount of gas formed in Mannite varies from a fourth to complete filling of the tube. In neutral red glucose agar shake cultures, gas is always shown by splitting of the media. Fluorescence is variable. Litmus milk is not clotted in ten days, and the primary acidity noted is permanent. Indol is *never* formed in peptone water as tested with the paradimethylamidobenzaldehyde and persulphate of potassium test after ten days incubation. We consider these tests sufficient in the case of an organism isolated from the blood. In our experience organisms giving all the above reactions in "sugars" and which are agglutinated microscopically at once by specific paratyphoid A serum occur in the fæces, but they are not necessarily paratyphoid A bacilli, and need further differentiation.

We differentiate such bacilli by the absorption method of Castellani, which we carry out as follows. A twenty-four hours growth of the suspected organism on agar is emulsified in about 0·2 cc. of paratyphoid A serum; the dilution of the serum is varied according to its *titre*, the object being to have an excess of bacilli for the amount of agglutinins present—for example, the *titre* of our serum is 1 to 300 and we use a 1 to 10 dilution of this serum. The emulsion is incubated for two hours at 37° C. and then centrifuged, the clear supernatant serum is put up in a series of dilutions in sedimentation tubes and its agglutinating power tested against our stock *B. paratyphosus* A. If the organism tested be *B. paratyphosus* A, then the specific agglutinins are completely removed. Before accepting a suspected organism we require that it shall completely remove the agglutinins specific for *B. paratyphosus*

A. As controls we have frequently tested heterologous organisms such as *B. typhosus*, *B. paratyphosus* B, and *B. coli*, against our *paratyphosus* A serum, but have never removed the specific agglutinins for *B. paratyphosus* A.

Serum Diagnosis.

As in enterica so in paratyphoid fever, the phenomenon of agglutination of the causal organism by the patient's serum is seen, but with, we think, a marked difference in degree, time of appearance, and permanency of the agglutinins. Antityphoid inoculation, as far as we have observed in some hundreds of cases, seldom if ever produces any agglutinins for *paratyphosus* A, with the result that a positive agglutination for this organism in a dilution of 1 to 20 is in our opinion very strong evidence of the nature of the patient's fever. A difficulty in diagnosis arises owing to the fact that if the serum of an inoculated patient is tested against *B. typhosus* alone, the agglutination *titre* is found to rise considerably, though the cause of the disease is the *B. paratyphosus* A, as proved by the isolation of this organism from the blood. Speaking generally, we would say that in an inoculated man suffering from paratyphoid fever, the original agglutination *titre* of his serum to *B. typhosus* due to his inoculation rises about the eighth day and reaches its maximum about the eighteenth, thereafter gradually declining during the next three months, whilst the agglutinins for *paratyphosus* A do not appear much before the twelfth day, reach their height about the twenty-fourth day, and totally disappear within two months.

Many authorities state that the organism which is agglutinated in the higher dilution by the patient's serum is the causal organism of the fever, and recommend this means of differential diagnosis between enterica and paratyphoid fever. In our opinion this will lead to a large percentage of errors in diagnosis, especially if only one or two observations are made. For example, we have seen cases of paratyphoid fever in which agglutination for *B. typhosus* was marked, and that for *B. paratyphosus* A absent even in 1 to 10 dilution throughout the disease (*vide* Chart No. 3). Again, we have met with cases in which the paratyphoid agglutinins never reached the high level of the enteric agglutinins, and still another type of case (severe) in which first the one and then the other organism agglutinated in the higher dilution (*vide* Chart No. 2). In this chart the .—.— line shows the agglutination curve for *B. typhosus*, and the ---- one that for *B. paratyphosus* A. In all these fevers the diagnosis was made by the isolation of the *B. paratyphosus* A from the blood.

In cases which showed agglutination both for *B. typhosus* and *B. paratyphosus* A we have attempted to demonstrate which was the specific agglutination as the result of the fever, and which was the group agglutination. Our method has been as follows: the patient's serum was diluted 1 to 5, and was divided into three portions. In one portion a thick emulsion of *B. typhosus* was made, in another, *B. paratyphosus* A was emulsified, and the third portion was kept as a control. These emulsions were left on the bench for twenty-four hours and then centrifuged. The agglutination power of the clear supernatant serum was then ascertained against the two organisms, this being also done with the control. We expected to find that absorption of the serum with *B. typhosus* removed all the agglutinins in the case of a convalescent enteric patient and *vice versa*, with the reservation that in the case of an inoculated man, absorption of his serum with *B. paratyphosus* A would not entirely remove the agglutinins for *B. typhosus* due to his inoculation. Unfortunately this test when applied in known cases of enterica or paratyphoid (proved by blood culture) gave results which were not in accordance with the theory, and hence we abandoned the test.

Epidemiology.

The question of the epidemiology of paratyphoid fever is one about which all that has been written with regard to enterica may be equally well applied. We have reason to believe that the disease is widespread in India (see map). The grounds for this belief are that we have been informed that fevers of seven to ten days duration occur in all the military divisions of India, and from our own observations on such fevers in the Lucknow and Meerut Divisions a large majority of them are paratyphoid fever. For example: During the last twelve months 126 blood cultures have been received in the Lucknow Divisional Laboratory for examination, and in spite of the fact that in many of the fevers the cultures should have been taken earlier, positive results were recorded in fifty-four cases. The *B. typhosus* was isolated thirteen times and the *B. paratyphosus* A forty-one times.

It is difficult to speak with any certainty of the protection afforded against this disease by our present anti-enteric inoculation. Theoretically, this should protect to some extent, but in practice, whilst enterica has decreased we find that since the introduction of modern methods of diagnosis (blood culture), a large proportion of cases hitherto returned as simple continued fever and pyrexia

of uncertain origin are, in reality, paratyphoid. What we mean by theoretically is, that if experiments be carried out on healthy men who are inoculated against enterica, we can demonstrate specific amboceptors to paratyphoid in apparently equal amounts to the specific amboceptors to *B. typhosus*, though the man had never had paratyphoid.

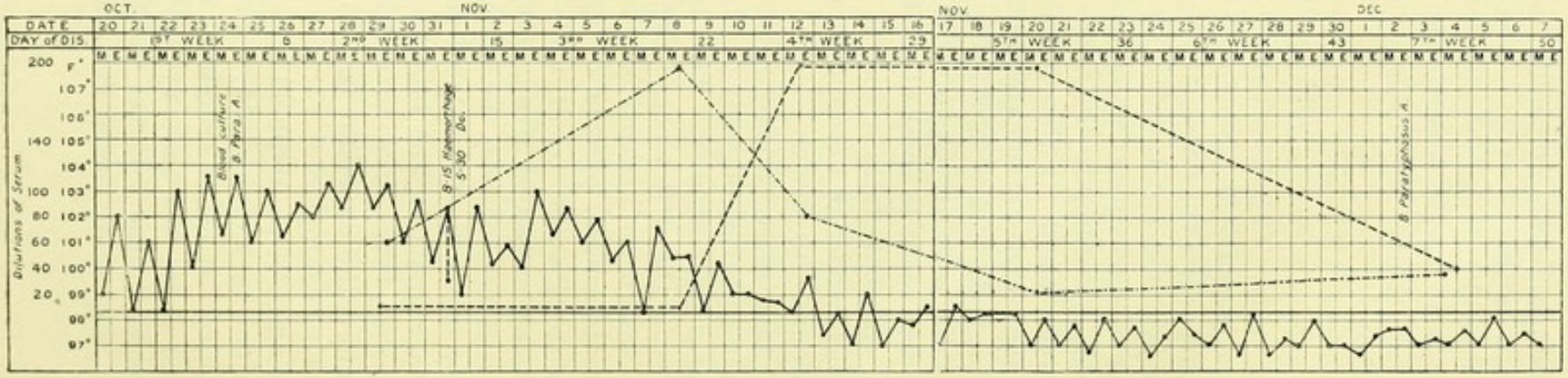
The Carrier.

By the term "chronic carrier" we mean a patient who continues to excrete the *B. typhosus* or *B. paratyphosus* for more than three months after the termination of his fever. During 1910-11, at Naini Tal Convalescent Enteric Depot, ten carriers were detected out of 157 convalescent paratyphoid cases. Only one of these carriers was chronic, according to the above definition, and this man ceased to excrete the bacillus within five months. It will be remembered that in enterica the chronic carrier occurs in about 2 per cent of all cases. Accounts of two outbreaks of paratyphoid fever in India, both of which were traced to acute carriers, have been published; reference was made to these at the beginning of this article.

From the above facts it will be noted that whereas the chronic paratyphoid carrier is uncommon, it is not so in the case of the acute carrier. An epidemiological feature which applies to paratyphoid much more strongly than to enterica is the comparative mildness of the great majority of the cases and the small death-rate as compared with enterica. This brings several interesting points into view; in the first place, owing to the mildness of the disease in some instances, patients are not diagnosed paratyphoid, but influenza, tonsillitis, pyrexia of uncertain origin, rheumatism or pneumonia, with the result that they are sent out of hospital within a few weeks of going sick, and may return to barracks while still infectious. In the second place, such convalescents are frequently sent with other debilitated men to the hills during the hot weather, and thus the hill stations where men of several corps mix together may serve as distributing centres of the disease. Furthermore, the ambulant case is a reality, and of such we give the following example.

Some cases of paratyphoid fever having occurred in a battery of Artillery during March, 1911, a preliminary attempt was made to pick out a carrier before examining each man *seriatim*. Every man was asked if he had recently had fever, and his medical history sheet was examined. It was noted that two men in room A and five men in room B looked debilitated without any apparent cause.

CHART 1.—To illustrate a mild case.



——·——·—— shows agglutination to *B. typhosus*
shows agglutination to *B. paratyphosus* A.

CHART 2.—To illustrate a severe case of Paratyphoid Fever.

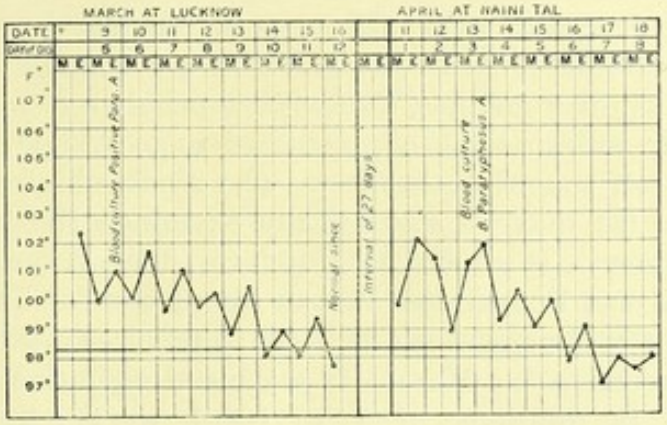


CHART 3.—To illustrate a mild case followed by a relapse, with negative Grüber-Widal reaction *B. paratyphosus* A, both during the primary attack and the relapse.

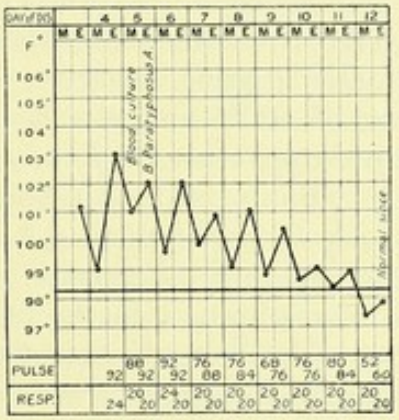


CHART 4.—To illustrate the common type of fever.

CHART 5.—To illustrate type of fever associated with cholecystitis in an acute carrier.

Their blood was therefore tested for agglutination against *B. paratyphosus* A, but with a negative result, on March 8. Between this date and March 20 the serum of every man in room B was tested in a similar manner, also with a negative result. We then passed on to examine the occupants of room A, and, by mistake, omitted to tell the medical officers, who kindly obtained the blood capsules for us, that we had already examined the blood of two debilitated men in this room on March 8. Thus it came about that the blood of one, Bombardier J., was tested a second time with an interval of twenty-one days between the observations. At the second observation on March 29 his blood agglutinated *B. paratyphosus* A in dilutions of 1 to 10, 1 to 20, and 1 to 40. Bombardier J. was sent for, and he then stated that he had felt ill for ten days; his symptoms being those which we associate with paratyphoid fever. He was sent to hospital, and thence transferred to Naini Tal Enteric Convalescent Depot. The examination of his excreta was commenced on April 11, and the *B. paratyphosus* A was recovered from his stools on April 11, 12, and 14; all subsequent examinations being negative. The organism gave the cultural reactions of *B. paratyphosus* A, absorbed all the agglutinins for *B. paratyphosus* A from specific serum, and furthermore, after injections into a rabbit, produced specific agglutinins for our stock *B. paratyphosus* A.

When investigating an outbreak, one is frequently struck by the apparent lack of any common factor in the immediate past history of the patients. To illustrate this point we give a list of men in a recent epidemic in the order in which they fell ill :—

Number	Patient	First day of illness	Regiment or Corps
1	Pte. H.	Feb. 11	8th Hussars.
2	,, B.	,, 21	King's Own, H. Company.
3	,, M.	,, 23	Highland Light Infantry, G Company.
4	,, T.	,, 24	King's Own, B Company.
5	,, T.	March 2	King's Own, Band.
6	Gr. S.	,, 2	U Battery, R.H.A.
7	,, J.	,, 2	,, ,,
8	,, R.	,, 3	,, ,,
9	Pte. W.	,, 4	King's Own, Band.
10	,, B.	,, 7	U Battery, R.H.A.
11	Dr. M.	,, 9	20th Battery, R.F.A.

Every European corps in the station was thus represented.

The difficulty of tracing the source of such an outbreak must be evident. What we think usually happens is as follows. An epidemic is started from one acute carrier, the nature of whose illness is not recognized owing to its mildness. He infects other

men, some of whom are recognized as cases of paratyphoid; others, again, are not diagnosed, and possibly others never report sick at all. These men soon return to barracks and come in contact with other men in soldiers' homes, &c., and, though probably only infectious for a few days, manage thus to disseminate the disease. In fact, we consider the acute convalescent carrier to be the chief cause in perpetuating the disease in India; and in this respect the epidemiological aspect of paratyphoid differs in degree from that of enterica, for in the latter disease the diagnosis is seldom mistaken, the man is isolated, and never returns to his corps till certified free from infection.

Effects of Sanitary Effort.

Outbreaks of paratyphoid have occurred among units in which there was a high degree of sanitary discipline (lines clean and well cared for, with latrines and urinals free from flies). Some medical officers are of opinion that the remarkable fall in the incidence of enterica that has taken place in India during recent years is largely due to the introduction into the British lines of the wet system of conservancy (saponified cresol). Where this system is properly carried out, the latrines and urinals are certainly free from flies and the trenching grounds no longer act as huge fly nurseries. Notwithstanding this satisfactory state of affairs, outbreaks of paratyphoid fever continue to occur. The incidence of enterica, which is becoming less and less each year, points to some condition or conditions at work which have a cumulative effect; we believe that these factors, in order of dominancy, are :—

(1) Segregation of the convalescent enteric patient, until he is proved to be free from infection.

(2) The elimination of the chronic carrier.

(3) Inoculation.

(4) General all-round improvement by attention to sanitary details.

The risk of the soldier contracting the disease is much greater in a large station than in a small one, owing to the constant fluctuations of the military population in the former; these fluctuations being due to athletic gatherings, rifle meetings, courses of instruction, and polo tournaments which are held in the larger stations and attract considerable numbers of men from other smaller cantonments.

Just as this paper was completed, we received the May number of the Journal of the Royal Army Medical Corps, containing

Major McNaught's article on paratyphoid fever in South Africa. His description of the clinical symptoms agrees closely with our own experience, so much so that it is difficult to withhold the conclusion that the paratyphoid fever of South Africa is the same as the paratyphoid fever of India. He states that the *B. paratyphosus* B is usually found in these South African cases. We confess that we do not understand the situation, as in all our experience in India we have not yet met with a case from which we have been able to isolate the B variety of this micro-organism. It is obvious that the whole question of the identity of the causative organism in these cases needs critical inquiry. It may be that workers who find the bacillus B in paratyphoid fever are not applying sufficiently accurate methods for differentiation and are relying too much on serum reactions. To discuss the fallacies underlying the serum reactions would make this paper too long, but we would remark that the apparent anomaly between our Indian experiences and those of observers in South Africa is a matter which we, in the Corps, might well attempt to elucidate.

When compiling this paper we have made full use of the records at the Naini Tal Enteric Fever Convalescent Depot, and of the observations of Major D. Harvey, R.A.M.C., for which we are greatly indebted. Our thanks are due to Major Blackwell, of the Corps, for numerous blood cultures, charts, and clinical observations ; we are also indebted to the Divisional Sanitary Officers, for their trouble in collecting information for us, while to Captain Gregg, R.A.M.C., we owe much for kindly help in the laboratory.

Summary.

We are of opinion that a considerable proportion, probably a third, of the cases returned in India as pyrexia of uncertain origin are in reality cases of paratyphoid fever due to infection by the *B. paratyphosus* A. What this means in extent of prevalence will be apparent when we add that no less than 4,386 cases of pyrexia of uncertain origin occurred among British troops in India in 1909. The highest incidence was during the months of April and May. Under the same heading in 1910, the cases dropped to 2,733.

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Original Communications.

GRANULE SHEDDING IN *TREPONEMA PALLIDUM* AND ASSOCIATED SPIROCHÆTÆ.

BY CAPTAIN W. R. O'FARRELL, R.A.M.C., AND ANDREW BALFOUR, M.D.
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IN the *Journal of the Royal Army Medical Corps* for June, 1911, one of us (A. B.) drew attention to the phenomenon of granule shedding in *Treponema pallidum* when this spirochæte is submitted to the action of salvarsan. A similar discharge of what are possibly spore-forms had been demonstrated in *S. granulosa penetrans* (n. sp.),[1] the fowl spirochæte of the Sudan. In its case the granule shedding occurs both naturally and under the influence of salvarsan. The occurrence of two cases of syphilis which came under the care of one of us (W. R. O'F.) has given us the opportunity of studying not only *T. pallidum* but other spirochætes associated with it in the primary lesion and we have found :—

(1) That granule-shedding in *T. pallidum* occurs before any treatment of the case is begun. It is, therefore, in all probability a feature in the life-history of the spirochæte. It is not so marked, however, as it is when salvarsan has been administered.

(2) That the same is true of other spirochætes associated with that of syphilis and is specially well seen in the case of *S. refringens*.

The following is an account of the cases mentioned :—

[1] Balfour, A. (April 1, 1911), "The Infective Granule in Certain Protozoal Infections, as illustrated by the Spirochætosis of Sudanese Fowls."—*British Medical Journal.*

The material for examination was obtained from two cases of syphilis.

Case No. 1.—A soldier, aged 23, first seen on May 21, 1911. Infection had taken place about three and a half months previously. He presented a clinical picture of the advanced secondary stage of the disease and might well be described as a case of florid syphilis.

The trunk and limbs were covered with an ecthymatous rash, the scalp was involved and the hair showed signs of epilation ; the *corona veneris* was well marked.

The left tonsil was ulcerated and the whole fauces and throat injected. The superficial lymphatic glands were generally enlarged, and the left inguinal chain showed signs of suppuration.

The chancre situated on the glans was typically indurated, there was slight phimosis and a purulent balanitis was present.

In the folds of the groin the skin was moist and ulcerated in places. The skin between the toes was ulcerated and the big toes showed signs of commencing onychia.

Skin Lesions.—A microscopical examination of the serum from the scraped surface of one of the ecthymatous patches, when examined by the dark background illumination method, revealed the spirochætæ of syphilis in considerable numbers. Careful observation showed certain of the parasites to be shedding small, highly refractile granules, and these latter were present in the medium and exhibited a certain amount of what appeared to be true motility. No other spirochætes were present.

On May 22, 0·6 grm. of salvarsan was injected intramuscularly, suspended in olive oil, the intra-scapular muscles being the site selected.

Examination of the Skin Lesion.—Four hours after the salvarsan had been given the skin lesion was again examined. The treponemata were still present, but they now presented a much greater activity than formerly, undergoing what has been described by one of us (W. R. O'F.) as the dog-shaking movement, an action associated with a rapid discharge of granules from the cell membrane, the granules racing up and down the length of the spirochætes and finally being, as it were, flicked off from one or other extremity of the parasite.

It was noted more than once that the granule was shot out some little way into the medium and this sometimes served to distinguish the spirochæte granule from other granules, probably of the nature of hæmiconia present in the film. The *T. pallidum* is so fine and pointed at the ends that the closest observation was required to make certain of this granule discharge, but it was witnessed by both

of us too often to leave any doubt in our minds as to what actually occurred. As a result of this granule shedding, certain of the spirochætes became very faint, lost their silvery appearance and eventually came to rest, either as wholly empty periplasts or as flattened cell membranes still containing one or two bright but motionless granules. Not infrequently such a granule was observed at one or other extremity as though the expiring spirochæte had just failed to expel it. There were also spirochætes which had been killed outright as an effect of the drug and appeared normal save for the lack of any motility.

May 23.—On this date blood-stained serum derived from the same skin lesion showed only free granules which from their size and motility appeared to be undoubtedly of spirochætal origin, blood granules, a few of these being the product of leucocytes, and small clumps of empty and motionless periplasts. No living spirochætes could be found.

May 24.—Only granules present. No signs of spirochætes or empty sheaths. On May 27, a similar result was obtained and by this time the skin lesion had greatly altered and was little more than a pigmented patch.

Examination of Chancre.—May 21. Before the administration of salvarsan a dark field preparation of slightly blood-stained serum from the lesion showed a vast number of spirochætes. *T. pallidum* was in evidence in well-nigh every field, but *Spirochæta refringens* was more common and exhibited great activity. In addition other species were present, some short and thick, others long and thin which we are unable to name; amongst them a *pseudo-pallidum* form was conspicuous, only to be differentiated from *T. pallidum* by its much greater motility and by the fact that its spirals were slightly better defined. As the result of prolonged observation it appeared to us that every one of the species of spirochætæ present was engaged in shedding granules. This phenomenon was of course best marked in the case of the large and comparatively stout *S. refringens*, the granules from which were brighter and larger, and possibly more active than those shed by *T. pallidum* and formed the majority of the varieties present.

May 22.—Four hours after the administration of salvarsan a film was put up and examined. The microscopic field presented an extraordinary appearance. The more active species of spirochætes were rushing about hither and thither, pausing, shaking themselves violently, and rapidly shooting out granules which went dancing about, the whole field soon becoming full of spirochæte granules

mixed with hæmiconia and other granules derived from the blood, those from the leucocytes frequently having a vesicular appearance which served to distinguish them from the other forms. The *T. pallidum* participated in the granule shedding, but in the crowded field it was not so easy to observe them in action as it was in the case of *S. refringens*. Very soon, in addition to granules, motionless periplasts could be seen together with spirochætes which had evidently been killed outright by the salvarsan, and still presented the usual silvery cylindrical aspect, but showed no sign of motility. At a later period a tendency to clumping on the part of the empty sheaths was noticed.

May 23.—A film examined on this date showed only one living spirochæte, a *S. refringens*, which was engaged in granule shedding. The granules were in evidence, and there were clumps of empty periplasts, those of *T. pallidum* being clearly distinguished from those of the coarser spirochætes.

May 24.—No *T. pallidum* found and no clumps seen. A few thick, sluggish spirochætes were found and seen to be granule shedding. It is believed that these were derived from the accompanying balanitis, the secretion from which contained on this day, as formerly, numerous spirochætes—*S. balanitis?*

May 27.—A scraping from the nearly healed chancre showed nothing but a few granules, the exact source of which it was impossible to indicate.

Stained Films.—The results obtained by the dark-field observations were confirmed by the examination of films stained by the Leishman and Giemsa methods, and by the Tamamato-Levaditi process. The Romanowsky-stained slides gave no evidence of granules either inside or outside the spirochætes. Possibly staining was not sufficiently prolonged, but there is no doubt that the free spirochæte granules have but a feeble affinity for polychrome methylene-blue and eosin.

Films treated by the silver process, on the other hand, showed up the granules well. The latter were stained a jet black and contrasted markedly with the brown or brownish yellow colour of the spirochætes which contained them, or near which they lay free. Not infrequently a spherical black granule was visible at one extremity of the parasite. The free granules are not always easy to distinguish from stain deposit, but some of the slides showed very little of the latter, and none of the dark spherical dots of precipitate stain, which closely resemble the infective granules.

Films made before and after the administration of salvarsan

also showed spirochætes staining darkly through their whole length, these being parasites which had either not started granule shedding, or had been killed outright by the drug. The clinical aspects of the case accorded well with the microscopical observations, and were briefly as follows :—

On the morning of May 23, the day following the salvarsan injection, the rash had faded off the thighs, the part least affected, the skin lesions and sore were remarkably vascular. On May 27, this vascularity had disappeared and the signs of the disease had nearly all vanished except for the pigmentation of the rash. The temperature between the 22nd and 27th had oscillated between 99° and 101° in the morning and evening respectively; between the 25th and 27th the temperature descended rapidly to normal. Whether this temperature, after the salvarsan injection, was due to constitutional disturbance, the effect of absorption of the drug, or the result of the granule discharge and possible liberation of toxins it is impossible to say, but the possibility of the last supposition must not be overlooked.[1] On June 5, all swelling, the result of the injection, had subsided, and the left inguinal glands were normal. The patient was discharged from hospital to attend for observation.

Case No. 2.—A soldier, aged 23, was first seen on June 3, 1911. Infection had taken place fourteen days previously. He presented an indurated sore on the prepuce with enlarged and indurated glands in the left groin.

On examining with the dark background illumination blood-stained serum obtained from scraping the chancre surface, the syphilis and other spirochætes were found to be present in large numbers, and some were observed shedding granules.

On June 4, the patient was given salvarsan. Four hours after the injection, serum from the chancre, when subjected to examination, was found to contain spirochætes, but a great many of these had been killed outright by the drug. A few were observed granule shedding; their action was, however, very feeble, and they did not appear to have strength enough to complete the expulsion of all their granules. A relatively larger number of granules were, however, present than on the previous day.

June 5.—Granules present, but no spirochætes or periplasts. The vascularity of the lesion was well marked.

These cases confirm the observations already mentioned as

[1] This pyrexia was not due to syphilis alone. Prior to the injection the temperature was only slightly raised above normal.

having been made on the effect of salvarsan on the *T. pallidum*, but they also show that granule shedding occurs independently of the action of the drug, and the phenomenon is not confined to the spirochæte of syphilis, but is one exhibited by the other spirochætes commonly associated with it in primary syphilitic sores. Salvarsan, however, greatly increases this granule discharge, which we still think is evidence of a protective action on the part of these spirochætes, undertaken by them with a view to prevent their total extinction, the granules being of the nature of resistant spores, the further history of which remains unknown, but which, so far as those derived from *T. pallidum* are concerned, doubtless play an important part in relapses, and in the later manifestations of syphilitic infections.

SOME OBSERVATIONS ON BODY TEMPERATURE.

By Captain P. DAVIDSON, D.S.O. and Captain N. DUNBAR WALKER.
Royal Army Medical Corps.

These observations were made by the officers specializing in physical training during their course at the Royal Army Medical College. All the temperature records, except those before rising, were taken in the rectum, with half-minute clinical thermometers retained for a full minute. All temperatures are Centigrade readings, and for convenience the corresponding Fahrenheit values are also given.

Many observers have drawn attention to the fact that a rise of body temperature occurs during muscular work and all are agreed that it is physiological, but that the rise of temperature may be of assistance to the body is not wholly understood. That the respiratory centre is rendered more sensitive by, and that the extensibility of muscle increases with a rise of body temperature are two points quoted in the textbooks. Lately Barcroft and King (*Journal of Physiology*, vol. xxxix) have shown that the dissociation of oxyhæmoglobin is facilitated by a rise of temperature, as "it appears that the oxygen dissociates from the hæmoglobin about twice as rapidly at 41°C. as at 36°C." ; they argue that this helps the mechanism for meeting the increase of metabolism in muscle which occurs during muscular work, because "each corpuscle spends a much shorter time in the capillary than when the muscle is at rest, and therefore it must divest itself of oxygen at a greater rate than is normally necessary." In "Further Advances in Physiology," Pembrey states: "It appears that a rise in temperature within certain narrow limits is beneficial. The chemical changes associated with muscular work are probably facilitated by a temperature a degree or two above the temperature during complete rest."

The point at which this rise may become pathological is of importance. In the "Second report on the physiological effects of food, training and clothing on the soldier" the committee state that "no abnormal results were observed when the rectal temperature of some men reached 38·8° C. (102° F.), but above that point inefficiency and a rapid irregular pulse were observed." The range of temperature in a normal man is less than 2° C. (3·5° F.) (Pembrey, "Animal heat, 'Schäfer's Physiology'"), and only once in our records

was this exceeded. It is probable that in the relationship of surface and deep temperature will be found an indication of impending pathological conditions, and it is in this direction that more work is required.

The results of eight marches carried out by five subjects are here charted and all particulars will be found in the tabulated diary.

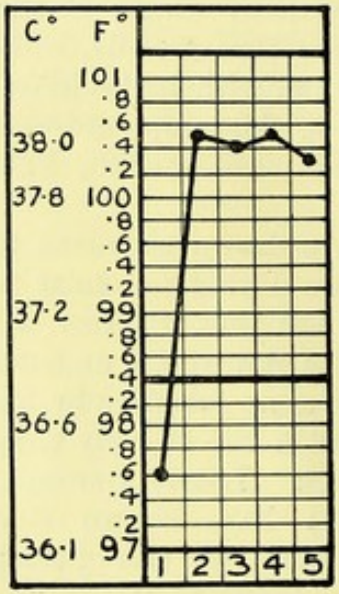


CHART 1.—The average rise for the five subjects for eight marches.

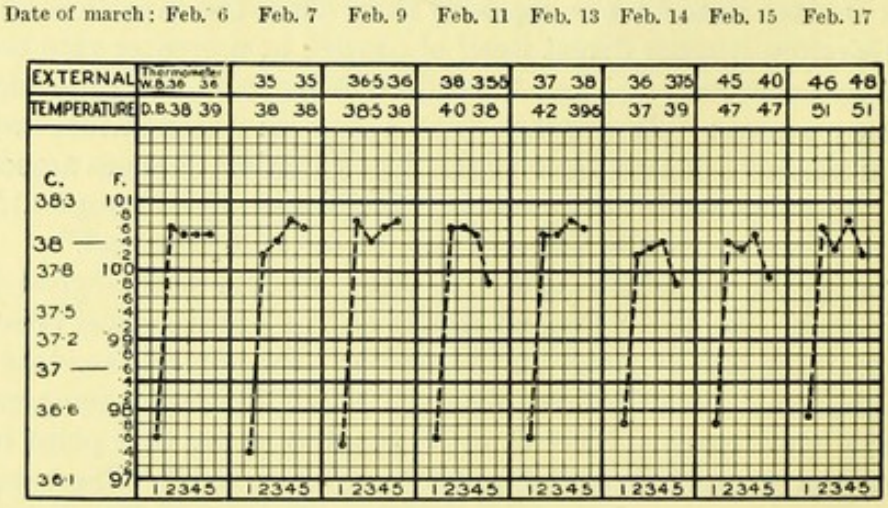


CHART 2.—The average rise of the five subjects during each of the eight marches.

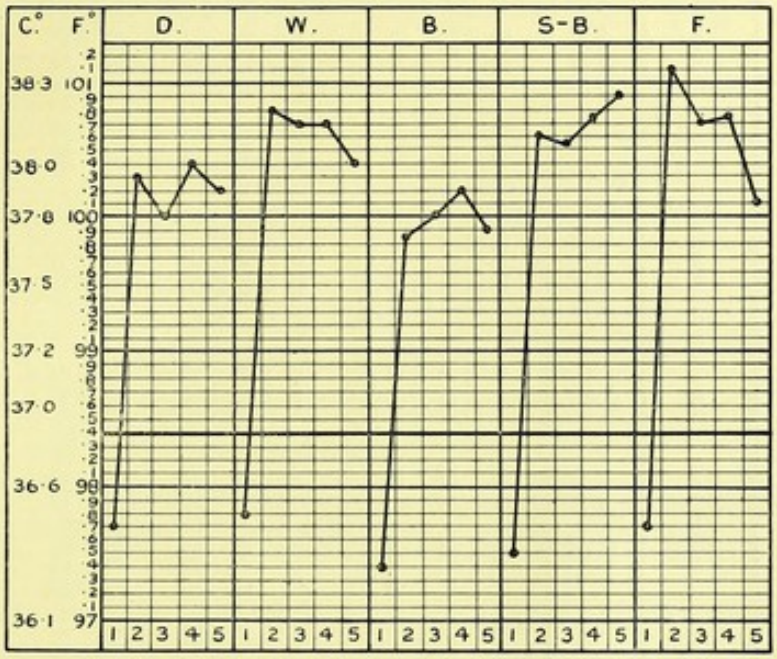


CHART 3.—The average individual hourly temperature during the eight marches.

(1) Temperature on rising.
(2) ,, at end of 1st hour's marching (10 minutes' halt).
(3) ,, ,, 2nd ,, (lunch halt, 30 minutes).
(4) ,, ,, 3rd ,, (10 minutes' halt).
(5) ,, on return home.

Mean weight of each subject (stripped) .. .. 68·2 kilos. (10 st. 10 lb.).
,, ,, ,, (loaded) .. .. 78·4 ,, (12 st. 5 lb.).
The average distance marched .. .. 24·4 kilometres (15¼ miles).
,, rate of march .. .. 98 metres per minute.
,, daily temperature .. .. .. 41·3° F. dry bulb. 38·4° F. wet bulb.
,, ,, relative humidity .. .. .. 77 per cent.

Individual age, height and weight (stripped) :—

D. ..	36½	years.	Height,	5 ft.	8¼	in.	Weight,	65·5 kilos.
W. ..	35	,,	,,	5 ,,	9½	,,	,,	72·7 ,,
B. ..	32	,,	,,	5 ,,	10½	,,	,,	67·2 ,,
F. ..	32	,,	,,	5 ,,	10¾	,,	,,	64·9 ,,
S.-B. ..	32	,,	,,	5 ,,	11	,,	,,	71·2 ,,

TABULATED DIARY OF ALL THE MARCHES.

Date	Time of start; hrs. and min. a.m.	Condition of roads	Weather	Thermometer				Total time in hours and minutes		Distance travelled		Rate of march, metres per min.	Work done. Calories per kilo. body weight		
				Start		Finish		Start to finish	Marching	Horizontal (km.)	Vertical (metres)		Horizontal	Vertical	Total
				W. B.	D. B.	W. B.	D. B.								
1911 Feb. 6 ..	10.33	Greasy and heavy	Dull and misty; breeze E.N.E.	36	38	36	39	4.41	3.51	22·5 (14 m.)	273·9 (913 ft.)	97·5	13·5	2·1	15·6
,, 7 ..	11.30	Dry and good going	Dull and misty; breeze S.W.	35	38	35	38	4.15	3.35	21·7 (13½ m.)	273·9 (913 ft.)	101·0	13·2	2·1	15·3
,, 9 ..	10.26	Dry, good going, except 3 miles very hilly	Dull and cloudy; no breeze	36	38	36	38	5.45	4·40	27·3 (17 m.)	478·3 (1,594 ft.)	97·7	16·4	3·6	20·0
,, 11 ..	10.43	Heavy and slippery	Clear and sunny	38	40	35	38	5.14	4.2	21·0 (13 m.)	434·4 (1,447 ft.)	90·0	12·4	3·2	15·7
,, 13 ..	10.39	Good, slippery in places	Clear and sunny	37	42	38	39	5.55	4.40	28·5 (17⅞ m.)	419 5 (1,398 ft.)	101·7	17·4	3·1	20·5
,, 14 ..	10.30	Slippery at start, later dry	Misty, then sun; breeze S.W.	36	37	37	39	5.37	4.18	26·2 (16 m.)	263·0 (877 ft.)	101·7	16·1	2·0	18·0
,, 15 ..	10.45	Slippery and muddy	Bright and clear; slight N. breeze	45	47	40	47	5.10	4.17	23·8 (14⅞ m.)	208·9 (696 ft.)	90·0	14·2	1·5	15·7
,, 17 ..	10.42	Very muddy and greasy in places	Bright and sunny; breeze N.W.	46	51	48	51	5.0	3·54	24.1 (15 m.)	455·9 (1,519 ft.)	103·2	15·1	3·4	18·5

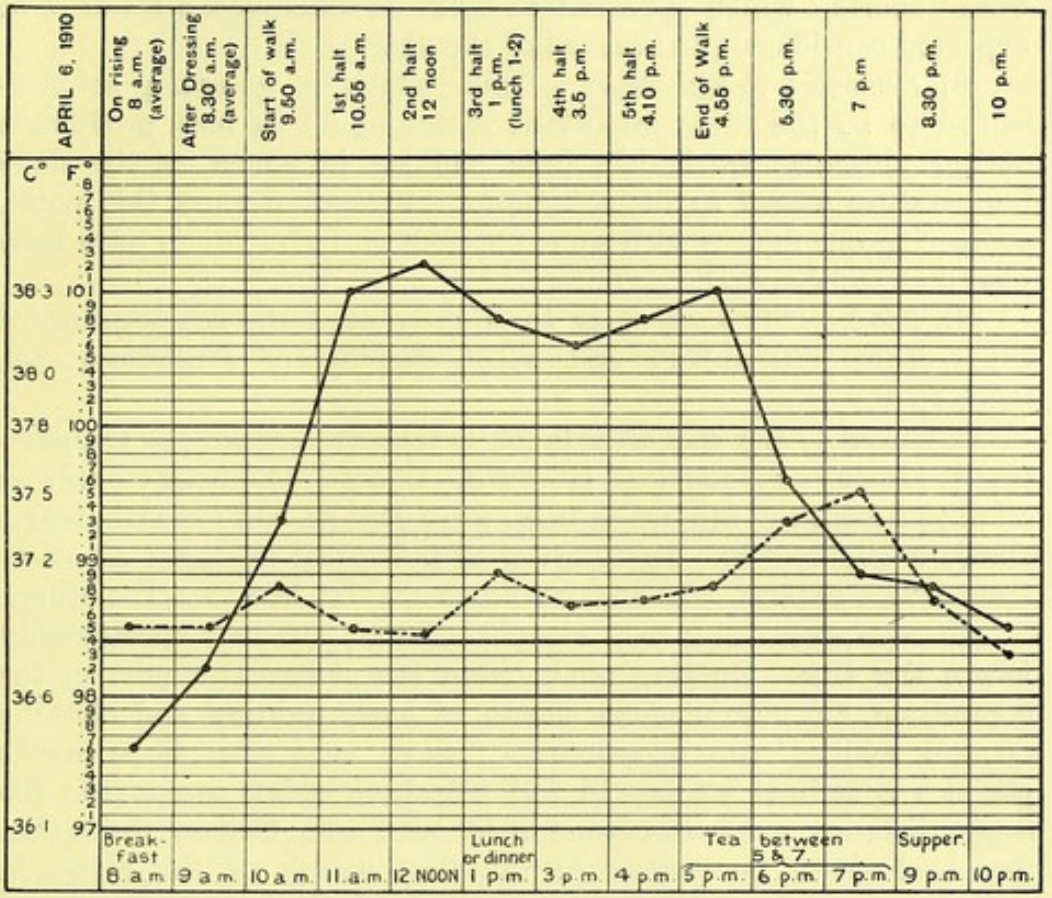


CHART 4.—Variation in body temperature throughout the whole day on which a long walk was taken. (Subject: N. D. W.)

Particulars of walk: Distance 32·18 kilometres (20 miles) at 97·5 metres per minute, no wind, cloudy and rain last 2 miles. Body weight 80 kilos, calories expended 1,704.

This chart is inserted to give some idea of the changes in body temperature before, during, and after a long walk. The unbroken-line curve, together with the hours above, represent height of and times of taking the temperature during the walk; the dotted-line curve and hours below are the average hourly variations per day of a medical student, representing some 343 observations. (Pembrey and Nicol, *Journal of Physiology*, vol. xxiii, 1898.)

Consideration of these records, in relation to the following :—

(1) *Work done.*—This has had no influence, as the amount performed did not vary greatly, although the rate of marching, an important factor in heat production, showed considerable variation. The average weight carried was 10 kilos. (22 lb.).

(2) *Clothing.*—All the subjects wore ordinary dress with rucksacs every day except on the 17th, when three (D., W., and F.),

wore "shorts," which appeared to make no difference, a result to be expected considering the season of the year.

(3) *External Temperature.*—This, which is generally acknowledged to be the most important factor of all in heat production during muscular work, has here had no influence, as the daily temperature varied so little. Later we shall discuss this point, comparing our figures with some obtained in India which have been placed at our disposal.

(4) *Food.*—"The effect is to slightly raise the temperature of the rectum." (Pembrey and Nicol, *Journal of Physiology*, vol. xxiii, 1898.)

In Chart 1, the rise after lunch is seen even when the temperature is already above 37·7° C. (100° F.). Chart 2 demonstrates this on all days except the 6th, when there is no change, and the 11th, which shows a slight drop. In Chart 3 of individual averages all show rises except subject W., who at lunch ate far less than the others.

(5) *Training.*—It is well established that this may considerably lessen the rise. Quoting again from the "Second report of the committee on physiological effects of food, training and clothing on the soldier," the experiments "show that after three weeks practice a trained soldier in full marching order can march the same distance over the same road with less fatigue than that produced when he performed the first march of the series in ordinary dress," as shown by a diminished rise of the rectal temperature. Also A. Mosso ("Life of Man in the High Alps," p. 132), summing up the results of some experiments with students climbing to a height of 400 metres, says "We see thus that training gradually lessens the increase of temperature after exertion." It is of course a well-known fact that with practice muscular work can be performed more economically and hence with less combustion, or as Mosso puts it, "Training is an unconscious instruction, which we give to the nervous system, which is thus taught to effect the contraction of the muscles without unnecessary expenditure of chemical work." All the subjects on this occasion had been living a somewhat sedentary life for the previous three months but had as a rule been accustomed to regular exercise. Here we find no reduction in the level attained in the later marches (see Chart 2); perhaps the number of marches was not enough to show this.

(6) *Fatigue* (see Chart 5).—On Febuary 9, the third march of the series, subject S. B., the only one of the party really fatigued, shows a high temperature at the finish which continued for some hours. This may have been due to delayed combustion of metabolites.

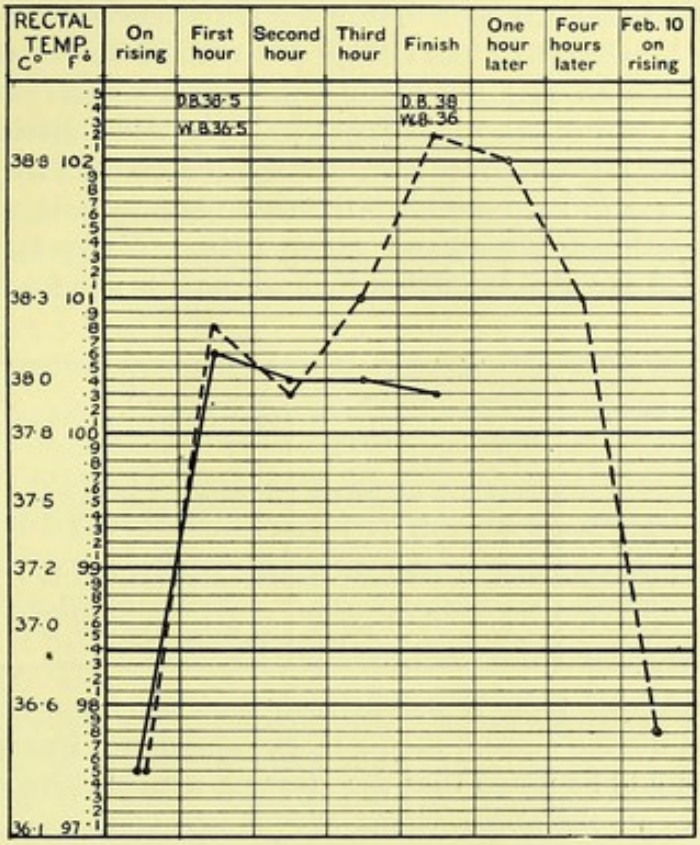


CHART 5.—February 9, 1911 : March of 27·4 km. (17 miles), rate of march 97·7 metres per minute.

Average of four subjects ; subject, S.—B. (dotted).

(7) *Individual Idiosyncrasies.*—As is well seen in Chart 3, some of the individuals attained their maximum temperature at the end of the first hour, while in others the temperature rose more slowly ; the level maintained throughout the march varied in different individuals.

General Conclusions.—It would appear, provided the heat regulating mechanism of the body is not interfered with by causes such as unsuitable clothing or a high wet-bulb temperature, that there is a " normal rectal temperature " for marching, in exactly the same way as there is a normal rectal temperature in bed after a night's rest. One might say that there is an optimum temperature for different amounts of body work, and in marching this appears to be somewhere between 37·8° C. (100° F.), and 38·3° C. (101° F.). Referring to Chart 1, we might go further and say that it was somewhere between 37·9° C. (100·2° F.) and 38.1° C. (100·6° F.). Similar observations to these made at the same time of the year in

1910 show that the average marching (33 observations) of three subjects was between 37·9° C. (100·3° F.) and 38·1° C. (100·5° F.).

One of us (N. D. W.) has made some further observations after bicycling and playing squash-racquets. They were obtained in the same way, using a one-minute Kew-certificated clinical thermometer retained for two full minutes. All these observations were made during the months of March and April, 1910. Walks taken during the same period are also tabulated and all records are for one hour's exercise.

Form of exercise	Clothing worn	Number of observations	Average temperature on starting	Average temperature after one hour	Difference	Remarks
			Degrees	Degrees	Degrees	
Walking ..	Ordinary dress	8	37·1 (98·8)	38·1 (100·5)	0·96 (1·7)	4 miles per hour
Bicycling ..	Ordinary dress	6	37·3 (99·1)	38·1 (100·6)	0·81 (1·5)	11 miles per hour
Squash-racquets	Flannels	8	37·7 (99·9)	38·5 (101·4)	0·81 (1·5)	Closed court

From the above table it will be seen that the highest temperatures occurred during squash-racquets, the maximum rise was 1·4° C. (2·4° F.). It should be noted that the starting temperature was high; this is because all the observations except one were made between 4 p.m. and 6 p.m., the time of the highest daily variation (Pembrey and Nicol, *Journal of Physiology*, vol. xxiii, 1898). It seems immaterial at what temperature one starts playing; on one occasion, starting at 10 a.m., it was found that the temperature rose in half an hour from 37·2° C. (99° F.) to 38·3° C. (101° F.). All the observations were made on separate days. The bicycling was over undulating country, and where more than one observation was made on the same day one hour's rest always intervened. In walking, each observation is that of a separate walk. The light clothing worn when playing squash-racquets and the rapid passage through the air when bicycling no doubt increased convection and evaporation, and hence the "difference"[1] is not so great as in walking, but we do not think the figures are comparable by estimating "difference." It would appear that what has been suggested about optimum temperature may also apply here and that for a more rapid and violent exercise such as squash-racquets a higher level is attained than in walking, to facilitate the more rapid chemical changes that are in progress.

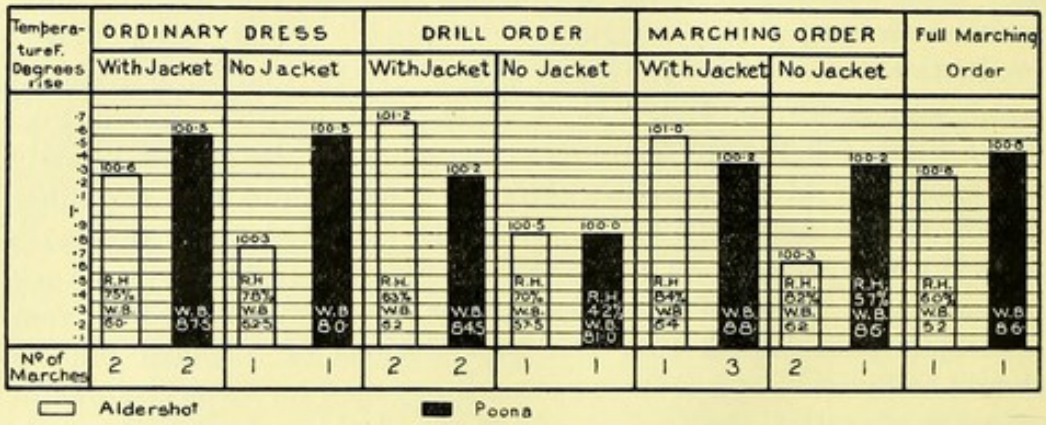
[1] "Difference" is the rise of temperature between the beginning and the end of the exercise.

To medical officers, this subject is of interest when considering the load carried and the clothing of the soldier when marching, and also the atmospheric conditions during marches. Zuntz and Schumburg ("Physiologie des Marches," p. 309), say: "Heat production is four or five times greater when marching than at rest," and again, p. 128, "It is interesting to note that a load of 31 kilos. (68 lb.) in favourable weather causes approximately the same rise of temperature (38°-39·7°) as a light one (22 kilos. or 48 lb.) in tropical heat." It should be noted these observers took the temperature in the urine stream. It is to prevent such physiological rises of temperature as we have recorded from becoming pathological that recommendations of medical officers should be directed and further research engaged in. The regimental officer now realizes the importance of the clothing, load, and march as discipline factors. The danger of the physiological temperature becoming pathological lies in "the fact that once the balance of the mechanism of heat regulation in the human body has been definitely upset by high external temperature, combined with almost total abolition of heat loss in evaporation, a vicious circle is established. The internal temperature rises, and as a result the oxidation processes and therefore the production of heat also increase, so that the body temperature rises still further, and so on." (Sutton, *Journal of Pathology and Bacteriology*, 1908, vol. xiii, p. 62). It is the difference between the dry and wet-bulb temperatures to which we wish to direct attention. Haldane (*Journal of Hygiene*, vol. v) in his broad conclusions says, "During muscular work in still air, the limit of wet-bulb temperature which could be borne without abnormal rise was much lower" (than when at rest). "At a wet-bulb temperature of about 87° F. (30·5° C.) the rectal temperature rose about 3.5° F. in an hour. In an air current of 135 linear feet per minute, a wet-bulb temperature of about 84° F. (29·5° C.) could be borne without abnormal rise of body temperature, but 87° F. (30·5° C.) was beyond the limit."

By permission of the Professor of Hygiene we have been allowed to examine Captain L. E. L. Parker's observations made in Poona, 1909. These have been compared with the results in the "Second Report on the Physiological Effects of Food, Training and Clothing on the Soldier," and are here charted and diagrammatically represented.

The diagram shows the rise of rectal temperature after marching two hours (about 7 miles) with a ten minutes halt at the end of the first hour. At Aldershot one of the marches (ordinary dress, with

jacket) was without a halt and another march (marching order, with jacket) was only of one hour's duration.



A comparison of the effect of different amounts of clothing and equipment on men marching at Poona and Aldershot. (Average of five men at Poona and four men at Aldershot.)

Captain Parker on both occasions superintended these marches and they differ only in the following points :—

Points of difference.	Aldershot	Poona
(1) Dress	Serge, with trousers	Drill with "shorts."
(2) Hour of starting	11 a.m.	8.30 a.m.
(3) Average starting body temperature	37·5° C. (99·45° F.)..	37·2° C. (98·95° F.)
(4) Average wet bulb temperature	60° F.	85° F.
(5) Relative humidity ..	73 per cent. (average)	58 per cent. Humidity (mean) at 8 hours for May, 1910, *Bombay Gazette* Supplement, Part II.

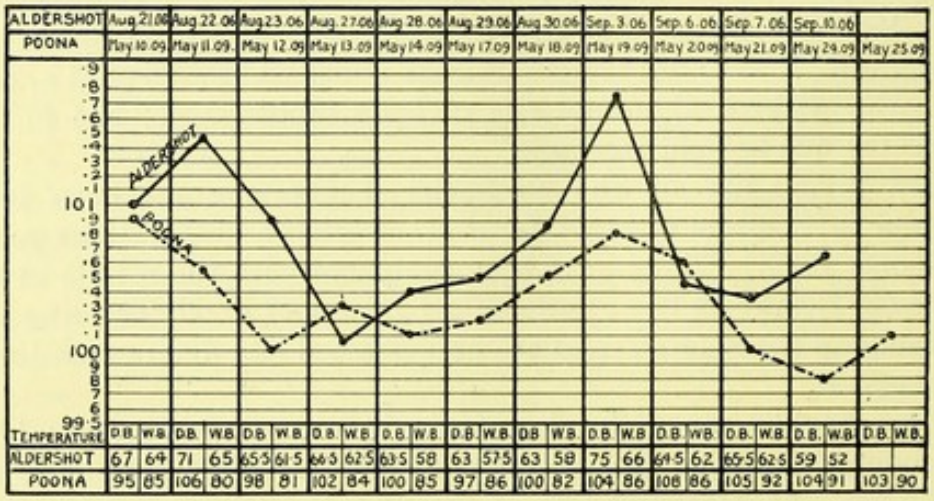


CHART 6.

The rectal temperature (average of four men at Aldershot and five at Poona) after marching two hours at Aldershot and Poona charted for comparison. The marches are recorded in sequence, irrespective of clothing worn and equipment carried. (See table below.)

There was a ten minutes' halt at the end of the first hour, except on August 21, when the march was only for one hour and on August 22 and 23, when there was no halt.

TABLE OF CLOTHING WORN AND EQUIPMENT CARRIED.

Date—1906	Aldershot	Date—1909	Poona
Aug. 21..	Marching order.	May 10..	Ordinary dress with jacket; no equipment.
,, 22..	In shirt sleeves, overcoat carried on the back.	,, 11..	Ordinary dress, no jacket or equipment.
,, 23..	Ordinary dress.	,, 12..	Drill order, no rifle or jacket.
,, 27..	,, ,, no jacket.	,, 13..	,, ,, no rifle.
,, 28..	,, ,,	,, 14..	,, ,,
,, 29..	Drill order, no jacket.	,, 17..	Marching order, no jacket or greatcoat.
,, 30..	,, ,,	,, 18..	Marching order, no greatcoat.
Sept. 3..	,, ,,	,, 19..	Full marching order.
,, 6..	Marching order, no jacket or overcoat.	,, 20..	,, ,, ,, with 100 rounds ball ammunition.
,, 7..	Marching order, no jacket.	,, 21..	Marching order.
,, 10..	,, ,, and 100 rounds ball ammunition.	,, 24..	,, ,,
		,, 25..	Ordinary dress.

NOTE.—The five men at Poona had all been in India from two to five years.

It is significant that the point to which the body temperature rose in Poona, with an average wet-bulb temperature approaching Haldane's limit (on the three last marches passing it) should not be greater than that at home in Aldershot, though the "difference" appears greater in the diagram on account of the starting temperature in India being lower, the earlier hour of starting possibly accounting for this. But in Chart 6, where the average march temperatures are recorded in sequence without comparing load or clothing, the lower level of body temperature maintained at Poona is very evident. With the lowered metabolism of Europeans in tropical climates, it might *a priori* be expected that the body temperature would be maintained at a lower level than in temperate climates; on this point the evidence is conflicting. Rattray (mouth), Davy (mouth), and Crombie (mouth and axilla) say it is raised. Boileau (axilla) confirmed by Thornley and Furnell, Pinkerton (axilla), and Wick (mouth), all maintain there is no change. Johnston (axilla) records an actual reduction. We can

find only one authority (Crombie) who has made any observations on rectal temperature in the tropics regarding this point, and he only states "that in healthy Europeans in India the rectal temperature is higher than that in the axilla." A series of rectal observations on new arrivals and those long resident in the tropics to clear up this matter would be most valuable. It should be noted that in the Poona experiments the difference between the wet and dry bulbs is very considerable, and that on no occasion did the atmospheric conditions approach saturation. At Aldershot the relative humidity was considerably higher than at Poona, and this with the difference in clothing at each station may partly explain the lower level maintained at Poona. It is of interest to note that with a thermometer below zero Linhard ["Danmark Expeditionem Til Grönlands Nordoskyst," 1906-8, vol. iv, No. 1 (see *British Medical Journal*, April 8, 1911)], says, "that on going for a walk with the thermometer 36° C. below zero the rectal temperature rose." These experiments in India tend to bear out our contention that there is an optimum marching temperature somewhere between 37·8° C. (100° F.) and 38·3° C. (101° F.) provided every facility is afforded to the natural means of heat regulation.

Our thanks are due to Captains G. A. K. Reed, W. W. Browne, R. H. Bridges, H. H. J. Fawcett, and C. R. Sylvester-Bradley for placing their results at our disposal and to Lieutenant-Colonel C. H. Melville for kind assistance with suggestions.

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EXPERIMENTAL KALA-AZAR IN THE GREY MONKEY OF THE SUDAN (*CERCOPITHECUS SABÆUS*).

By Lieutenant W. E. MARSHALL.
Royal Army Medical Corps.

(*From the Floating Laboratory of the Wellcome Tropical Research Laboratories, Khartoum.*)

Successful experimental inoculation of animals with kala-azar has become a well-recognized fact in the case of the parasite of infantile kala-azar (*Leishmania infantum*). Nicolle and his colleagues, pioneers in this work, have frequently infected monkeys and dogs. So far they have only succeeded in infecting these animals by intraperitoneal inoculation, and subcutaneous injection has only given a local reaction without general infection. They also found that passage of the disease from monkey to monkey attenuated the virus, but the same phenomenon did not occur in dogs. They recognize differences in the infection in these two animals. In the monkey there is marked enlargement of the spleen, and the disease resembles the human type of the disease. In the dog the spleen is only slightly enlarged and the infection is insidious. One of the dogs inoculated with the disease, and which died seventeen months after inoculation, showed no symptoms for fifteen months. They also found that in a series of dogs there was great variation in the susceptibility of the individual animal. With regard to immunity they have found that in an animal incompletely cured, or only just recently recovered, a second inoculation produces a severe and fatal result, whereas in an animal cured for some time immunity is conferred. Immunity is also produced by the virus of Oriental sore. Excision of the spleen in the dog has produced no effect on the course of the disease.

Nicolle and his colleagues have also found that Leishmaniosis occurs as a natural infection in the dog, and this has recently been confirmed by Gabbi and Basile in Italy, Critien in Malta, Alvarez in Lisbon, and the Sergents in Algiers. Basile recognizes two different forms of natural infection in the dog, the acute and chronic, and he thinks that the former, which affects principally young dogs and lasts from three to five months, plays a more important part in the spread of the disease. He was able to infect three young dogs by keeping them beside cases of infantile kala-azar, and he thinks the flea (*Pulex serraticeps* and perhaps

P. irritans) is the likely transmitting agent. Jemma, Gabbi, and Alvarez and Pereira da Silva have all succeeded in infecting dogs intraperitoneally with *L. infantum*, and Jemma has also produced infection by intravenous inoculation.

Laveran and Pettit, besides infecting dogs, have tried infecting rats and mice, but could not produce a general infection.

Novy, by the use of large and repeated doses of cultures of *L. infantum* on Novy and MacNeal's medium, has been able to produce infection in the dog.

Patton, working with Indian kala-azar, failed to infect dogs even with repeated inoculation, but probably he killed his animals too soon to say definitely that infection does not occur.

Present Investigation.

The following experiments were carried out with parasites obtained from cases of kala-azar in Sennar Province in the Anglo-Egyptian Sudan. In this province the disease affects chiefly children about 12 years of age, though adults are also occasionally infected. So far we have found the best method of infecting monkeys is from a splenic puncture during life. An ordinary sterilized hypodermic syringe is taken, washed out with sterile citrate solution, and spleen puncture carried out in the ordinary way, the contents of the syringe being immediately injected into the peritoneal cavity of a monkey.

Twelve monkeys were used in these experiments, and the results are shown in the following table. Eight monkeys were infected with kala-azar, in three the experiment failed, and in one the result still remains doubtful.

Infected Cases.

Of the eight infected cases, five were infected intraperitoneally, one subcutaneously, one subcutaneously and intravenously, and one was naturally infected.

Of those infected intraperitoneally, monkey B was chloroformed on the 62nd day, when the spleen was found to be enlarged, and Leishman bodies were present in moderate numbers; no parasite could be found in the liver, so probably the spleen becomes infected before the liver.

Monkey D died on the 65th day from a mixed infection of tuberculosis and Leishmaniosis, and it is interesting to record the degenerate nature of the parasites in this case. The parasites

were most degenerated in the spleen, which was heavily infected with tubercle, and were best preserved in the bone-marrow, where the tubercles were scanty. The post-mortem examination was done immediately after death, so the change in the parasites could not be a post-mortem one.

Monkey E was infected by the 54th day, became very ill with diarrhœa on the 143rd day, and was chloroformed on the 145th day. It was heavily infected with parasites.

Monkey N, inoculated intraperitoneally from monkey E, remained quite well, but, owing to Leishman bodies being present in monkey S (*vide infra*), liver puncture was carried out, and doubtful Leishman bodies were seen 35 days later, the 155th day; the monkey died, and the post-mortem examination showed the spleen to be enlarged, and parasites were found in the spleen, liver, and bone-marrow. There was a small ulcer in the ileum.

Monkey P, which died on the 134th day, was heavily infected with parasites.

Of those infected subcutaneously, in the case of monkey R the injection was made into the subcutaneous tissues of the thigh with a post-mortem splenic emulsion from a case of kala-azar. On the 150th day the spleen was found to be enlarged, and liver puncture showed the presence of Leishman bodies. On the 156th day the peripheral blood was examined, and in the fifth slide three parasites were found inside a large mononuclear cell. This monkey is still alive.

Monkey L received an intravenous and subcutaneous injection; it was intended to inject intravenously, but only the first portion of the virus entered the vein, the remainder entering the subcutaneous tissues of the forearm. Examined on the 161st day the spleen was found to be enlarged, and spleen puncture showed the presence of Leishman parasites. This monkey is also still alive.

The remaining monkey, which was a very young animal, was put in the same cage as monkey N on the day that the latter was inoculated intraperitoneally from monkey E. It remained in that cage, and on the 112th day it became ill. On the 117th day liver puncture showed the presence of Leishman bodies, and on the 121st day it died, thirty-four days before monkey N. It was very heavily infected, parasites being present in large numbers in the spleen, liver, and bone-marrow.

With regard to the negative cases, monkey H was inoculated with a splenic emulsion from a case of kala-azar, the emulsion being made eight hours after the death of the patient, when no

Animal	Where inoculated and date of inoculation	Material inoculated	Course of disease	Result	Organs infected	Remarks
Monkey B..	Liver and peritoneum 9.1.10	Post mortem spleen puncture. Case 4	30th day liver puncture negative — 53rd day liver puncture negative — 62nd day chloroformed	Infected	Spleen + Liver −	Spleen slightly enlarged; parasites present in moderate numbers.
Monkey C..	Peritoneum 12.3.10	Post mortem spleen emulsion. Monkey B	8th day peripheral blood negative — 22nd day peripheral blood negative — 33rd day peripheral blood negative — 45th day peripheral blood negative. Liver puncture negative — 66th day liver puncture negative — 94th and 95th days liver puncture negative — 108th day chloroformed	Not infected	Spleen − Liver − Bone-marrow −	
Monkey D..	Peritoneum 21.4.10	Spleen puncture. Case 3	14th day peripheral blood negative — 55th day liver puncture negative — 63rd day Ill.: Dyspnœa, splenomegaly. Liver puncture negative. Spleen puncture negative — 65th day died	Infected	Spleen + Liver + Bone-marrow +	Miliary tuberculosis also present; spleen markedly infected with tubercle.
Monkey E..	Peritoneum 23.4.10	Spleen puncture. Case 1	14th day peripheral blood negative — 54th day liver puncture positive — 58th day peripheral blood negative — 78th day Spleen enlarged. Spleen puncture positive. Liver puncture negative. Peripheral blood negative — 143rd day diarrhœa. Liver puncture positive — 145th day chloroformed	Infected	Spleen + Liver + Bone-marrow +	Monkey emaciated; spleen enlarged; liver enlarged and cirrhotic; bone-marrow red; intestinal intussusception present.
Monkey H..	Peritoneum 21.6.10	Post mortem spleen emulsion. Case 1	65th day chloroformed	Not infected	Spleen − Liver − Bone-marrow −	The spleen emulsion in Case 1 was made 8 hours after death.

Monkey K..	Sub-cutaneous tissues 11.7.10	Spleen puncture. Monkey E	160th day spleen enlarged. Liver puncture negative	161st day spleen puncture negative	163rd day spleen puncture negative		—	—	Still alive.
Monkey L..	Intra-venous and sub-cutaneous 11.7.10	Spleen puncture. Monkey E	161st day spleen enlarged. Spleen puncture positive				Infected	—	Still alive.
Monkey N..	Peri-toneum 11.7.10	Spleen puncture. Monkey E	120th day liver puncture doubtful		155th day died		Infected	Spleen + Liver + Bone-marrow +	Small ulcer in small intestine, ileum ; smears and sections of this negative.
Monkey O..	Peri-toneum 26.7.10	Post mortem spleen puncture. Case 10	42nd day found dead				Not infected	Spleen – Liver – Bone-marrow –	Spleen not enlarged.
Monkey P..	Peri-toneum 31.7.10	Post mortem spleen emulsion. Case 11	123rd day liver puncture positive		134th day died		Infected	Spleen + Liver + Bone-marrow +	—
Monkey R..	Sub-cutaneous tissues 31.7.10	Post mortem spleen emulsion. Case 11	150th day spleen enlarged Liver puncture positive		156th day peripheral blood positive		Infected	—	Still alive.
Monkey S..	Not inoculated	Kept in cage beside Monkey N	112th day ill	117th day liver puncture positive	121st day died		Infected	Spleen + Liver + Bone-marrow +	Lived continuously with Monkey N ; became naturally infected, and died before Monkey N.

parasites were visible in the spleen smears (monkey E was successfully infected from the same case during life).

Monkey C was inoculated with a spleen emulsion from monkey B, but was not infected by the 108th day, when it was chloroformed. Probably, as Nicolle has pointed out, the parasite tends to lose its virulence when inoculated from monkey to monkey, and this might account for the non-infection in this case, though the positive results obtained in monkeys L and N show that infection can be conveyed from monkey to monkey. In the case of monkeys L and N, however, the inoculation was made from a splenic puncture during life, and in the case of monkey C a post-mortem splenic emulsion was used.

Monkey O died 42 days after inoculation. There was no apparent cause of death, and no Leishman bodies were found.

Monkey K, which was inoculated subcutaneously, and which is still alive, shows definite enlargement of the spleen, but one liver puncture and two spleen punctures have so far given negative results.

The Presence of Parasites in the Peripheral Blood of Infected Monkeys.—Parasites are present in the peripheral blood of infected monkeys, but are difficult to find. In two infected monkeys, still alive, we have searched carefully for parasites in the peripheral blood. In one monkey we found in the fifth slide three parasites inside a large mononuclear cell; in another monkey, out of seven slides examined, we found one parasite inside a polynuclear cell.

Captain Archibald, R.A.M.C., has also successfully infected the red monkey of the Sudan (*Cercopithecus ruber*) with the disease.

We have tried inoculating dogs, but so far have failed to infect that animal as, with one exception, all the animals died soon after inoculation. We have also not yet met with spontaneous Leishmaniosis in the dog.

In the Sudan form of kala-azar the parasite is present in the peripheral blood in over 80 per cent of the infected people. The parasite morphologically resembles that of Indian kala-azar and infantile kala-azar; growth into flagellate forms has been obtained in 10 per cent. citrate, on Novy and MacNeal's medium, and on Nicolle's modification of that medium. Full details of these investigations will appear in the reports of the Wellcome Tropical Research Laboratories at Khartoum.

Conclusions.

(1) The ordinary grey monkey of the Sudan, *Cercopithecus sabæus*, can be infected with kala-azar.

(2) It can be infected by intraperitoneal or by subcutaneous inoculation of the parasite.

(3) It can also be infected naturally, an uninoculated monkey, living in close contact with an infected monkey, having contracted the disease.

(4) The parasites are present in the peripheral blood of infected monkeys.

(5) So far we have found that the best method of infecting the monkey is by injecting into the peritoneal cavity the contents of a spleen puncture taken during life.

I beg to acknowledge my indebtedness to Captain D. S. B. Thomson, R.A.M.C., who was associated with me in investigating kala-azar in Sennar Province, for help and advice, and to Captain R. G. Archibald, R.A.M.C., for carrying on the experiments while we were both absent on leave.

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CHRONIC BACILLARY DYSENTERY DUE TO A HITHERTO UNDESCRIBED BACILLUS.

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Although bacillary dysentery has been attributed at various times to many other members of the coli group besides the Shiga, Kruse, and Flexner bacilli, I have thought it would be useful to put on record the following case which seems to have been caused by a bacillus which, so far as I have been able to trace, has not been previously described.

The patient, a man aged 35, was taken ill whilst in Morocco in September, 1909, with a disease characterized by fever, diarrhœa, and the passage of blood and mucus; he was extremely ill at the time, and from his statement the symptoms were those of a pretty severe attack of dysentery. This first illness lasted for about two weeks, after which he recovered sufficiently to resume his work; but between that time and the end of 1909 he had two acute relapses, each lasting three or four days, during which he passed blood and mucus. From January, 1910, until the time he consulted me at the end of April in the same year he suffered continually from dyspepsia, irregularity of the bowels, with frequent diarrhœa and the passage of quantities of mucus, occasionally bloody, and his general health became seriously impaired. When I first saw him the patient was pale, sallow, and emaciated, he was easily exhausted, and his general aspect was that which is so common in cases of chronic tropical diarrhœa; his appetite was poor and capricious, and he was passing three or four watery offensive stools daily; there was a good deal of abdominal pain of a griping character, but he did not suffer from tenesmus. The tongue was coated with a thick yellow fur, and there was an area of marked tenderness over the splenic flexure of the colon, but no thickening of the gut could be felt. A white cell count of the blood showed 9,800 leucocytes per cubic millimetre, with no alterations from the normal in the percentage of the different white cells present. His serum failed to agglutinate laboratory strains of Flexner's and Shiga's bacilli or Paratyphoid B, but it agglutinated the bacillus which was isolated from his stools, and which will be described later, in a dilution of 1 in 60 in an hour. The stools were

watery, extremely offensive, and contained numerous fragments of undigested food and a considerable quantity of mucus; there were a number of polymorphonuclear leucocytes present, but no blood cells or amœbæ were found either at the first or subsequent examinations. Culture of the mucus on neutral red bile salt lactose agar gave an almost pure growth of an apparently non-lactose fermenting organism the details of which will be given below. *Bacillus coli* was conspicuous by its absence. Treatment consisted in putting the patient on a milk diet with 4 oz. of mutton or chicken daily, and a small quantity of bread; in addition, he was given 10 oz. of a milk culture of Massol's bacillus daily. The colon was irrigated daily with two litres of saline solution, followed as soon as this was evacuated by two litres of a 1 in 1,000 solution of quinine hydrochloride in saline which the patient was instructed to retain as long as possible. The irrigating solutions were run into the bowel through a long tube from a height of 18 in., the temperature of the fluid being 103° F. in the can. In addition, considering that the bacillus which was present in such numbers in his stools seemed to be the cause of the trouble, he was given weekly doses of 100 millions of a vaccine made from it.

Under this treatment he very rapidly improved, the diarrhœa was replaced by constipation requiring castor oil, mucus very soon ceased to be found in the stools, and the irrigation was discontinued at the end of two weeks. The organism referred to, however, continued to be present in the fæces to the exclusion of all *B. coli*, until May 14, when cultures failed to give any growth at all (this result may have been due to the fact that the sample was collected after the quinine irrigation). On May 21 the cultures showed large numbers of *B. coli*, with only six colonies of the non-lactose fermenting organism on two 12-in. plates. On May 28 the stools appeared to be perfectly normal, there was no mucus, the fæces were almost free from smell, and gave a copious growth of *B. coli*, with only three non-lactose fermenting organisms on two plates. The patient's general health had improved very much, and his subsequent convalescence was uneventful; at the last observation of the fæces no non-lactose fermenting colonies were to be found.

Characters of the Organisms in the Fæces.—Of a very large number of colonies which were tested, some twenty or thirty, all but one corresponded to the characters described below; the one exception was a colony which proved to be composed of Flexner's bacillus; it was found at the first examination and never subsequently. The other organism produced colonies resembling

those of *B. typhosus* on neutral red lactose bile salt agar; it was a non-motile rod, about as long as a typhoid bacillus, but somewhat thicker, growing isolated, but occasionally seen in chains of two or three elements. It did not stain by Gram's method, and did not liquefy gelatine. Culture in broth gave a diffuse growth, litmus milk became acid in twenty-four hours, and the acidity was permanent. In the first experiments with the freshly isolated culture there was no clotting of the milk, but in later tests with the culture, after it had been subcultured on agar for about a month, the milk became clotted on the tenth day, indol was formed in peptone and salt solution within five days. The bacillus produced acid and gas in glucose and maltose media within twenty-four hours, and in dulcite media in three days. Sucrose, mannite, raffinose, inulin, and salicin were unchanged after fifteen days; there was no fluorescence produced in neutral red glucose bile salt agar. The reactions with lactose were somewhat peculiar, the original colonies had all the appearance of those of a non-lactose fermenting organism, and with the earlier cultures lactose was unchanged after fifteen days. But on again testing the same strains, after several subcultures on agar, it was found that the bacillus had acquired the power of fermenting lactose; in these later tests there was no change until the end of the seventh day, after which acidity slowly developed and was marked by the tenth day; no gas production occurred in the lactose cultures. The results of simultaneous tests, made on the same batch of media to compare the organism with Morgan's bacillus No. 1 (A), and the bacillus of Hog cholera (Macfadyean), are given in the accompanying table :—

	Patient's bacillus				Morgan 1 A				Hog cholera			
Days	1	3	7	14	1	3	7	14	1	3	7	14
Litmus milk..	A	A +	Bleached	Clot	A ±	Alk.	Alk. +	Alk. ++	A ±	A	A	A ±
Glucose ..	A G	A G	A G	A G	A G	A G	A G	A G	A G	A G	A G	A G
Lactose ..	–	–	A ±	A	–	–	–	–	–	–	–	–
Sucrose ..	–	–	–	–	–	–	–	–	–	–	–	–
Mannite ..	–	–	–	–	–	–	–	–	–	–	–	–
Maltose ..	A G	A G	A G	A G	A G	A G	A G	A G	–	–	–	–
Dulcite ..	–	A G	A G	A G	–	–	–	–	–	–	–	A ±
Raffinose ..	–	–	–	–	–	–	–	–	–	–	–	–
Inulin ..	–	–	–	–	–	–	–	–	–	–	–	–
Salicin ..	–	–	–	–	–	–	–	–	–	–	–	–
Neutral red ..	–	–	–	–	–	–	–	–	–	–	–	–
Indol	..	..	+	+	..	..	+	+	..	..	–	–

It will be seen that the bacillus differs from Morgan 1 A in producing permanent acidity and eventual clot in milk, that it

ferments lactose with the formation of acid after seven days, also that it produces acid and gas in dulcite media. It differs from the bacillus of Hog cholera (Macfadyean) in producing the late clotting of milk, in forming acid and gas with dulcite, also in the fact that, like Morgan 1 A, it forms indol.

The bacillus was tested for its pathogenic properties on rabbits and guinea-pigs. In doses of ½ c.c. of a twenty-four hour broth culture given hypodermically to rabbits, it killed the animal within eighteen hours with symptoms of diarrhœa. Post-mortem: there was a large gelatinous exudate at the point of inoculation, with general and severe congestion of the vessels of the abdominal wall and numerous subcutaneous hæmorrhages; there was a quantity of blood-stained serum in the peritoneal cavity, and the whole length of the small intestine was acutely inflamed. The large intestines and cæcum were much distended with gas, but were not inflamed. The spleen was not enlarged. Cultures from the heart blood gave a copious growth of the organism. On the other hand, the same culture administered to rabbits by the mouth in repeated doses of 2 to 5 c.c. produced no symptoms whatever, and similarly, a dose of 10 c.c. of a filtrate of a ten-day old broth culture gave no result when administered hypodermically to rabbits. The virulence of the organism was lost after a month's sub-culture on agar. In guinea-pigs a hypodermic inoculation of 5 c.c. produced a large induration at the point of injection, which subsequently gave way to a large slough; there was no diarrhœa, and the general health of the guinea-pigs was not impaired. The culture of Flexner's bacillus, which was isolated on one occasion, produced no effect on either rabbits or guinea-pigs, whether injected hypodermically in a dose of 5 c.c. of the broth culture, or administered by the mouth.

My reasons for thinking that the organism which has been described was probably the cause of the patient's illness are: It was a pathogenic bacterium having a special affinity for the bowel, and was present in enormous numbers in the stools and especially in the mucus; it was agglutinated by the patient's serum in a dilution of 1 in 60, and not by normal sera in dilutions above 1 in 5; its decline in numbers and eventual disappearance from the fæces coincided with the improvement in the patient's general and bowel condition. It is justifiable also, I think, to believe that the rapid improvement after administration of a vaccine made from the bacillus was some evidence that it was this germ which was causing the trouble; it might be retorted to this, however, that

the other treatment which the patient received was the cause of his improvement. I can only answer that the patient was practically free from bowel symptoms a fortnight after the commencement of treatment, an unusually rapid progress in cases of chronic bacillary dysentery treated on general lines without vaccine.

My reasons for excluding the idea that the case was one of infection with Flexner's bacillus were: the patient's serum did not agglutinate this germ, and the single colony of Flexner's bacillus which was found was not pathogenic. An interesting point in the reactions of the bacillus is that after sub-culture it developed some power of fermenting lactose and clotting milk, and that its activity in fermenting maltose and dulcite was much increased after living on artificial media for a month.

INCINERATION IN CANTONMENTS IN INDIA.

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Cantonment incineration presents many inherent difficulties which do not hamper the practice of this conservancy method in the field, and, in view of these special difficulties, the whole problem calls for re-consideration as we face afresh the familiar questions :—

(*A*) Is incineration practicable in cantonments?

(*B*) Will it increase the cost of conservancy?

(*C*) Will its adoption increase military efficiency?

A. Practicability.

Unfortunately this is not merely a matter of physics—rather is it a question dominated by the obstructive æsthetic, the myopic economist and the unspeakable *mehtar*. We want to know that we can incinerate excreta without causing undue offence, with the material at hand and without running up the conservancy bill, and without overtaxing the intelligence and reliability of the sweeper.

The hygienic desiderata are that potentially infective dejecta should be rapidly destroyed, while being protected meanwhile from all disseminating agencies, especially from flies which incidentally should be reduced to minimal numbers.

(1) *Disposal of Urine.*

The matter would be reduced to comparative simplicity if some means could be devised for safely disposing otherwise of the great bulk of the 300 odd gallons of urine passed daily by 1,000 men. Various methods have been adopted or suggested :—

(*a*) *Distant Trenching.*—If solids are to be burnt and urine trenched, it is clear that maintenance of this dual organization must militate against economy. Moreover, cartage of urine alone will lead to dumping of considerable quantities on cantonment outskirts, which could not occur if the carts contained mixed filth.

(*b*) *Surface Irrigation after Heat-Sterilization.*—No suggested device has provided automatic security against the chance of possibly infective urine being run, unsterilized, on to the surface soil of cantonments. Hitherto strong exception has been taken to this method, and especially so in stations deriving their water-supply from shallow wells in or near the lines.

(*c*) *Disposal in Buried Soakage-pits.*—It is suggested that urine

from the urinal trough be piped direct through a buried iron lid into an underground pit filled with cinders. This certainly prevents dissemination of urinary organisms by flies and wind, but other aspects require very careful consideration.

On the one hand we know that *B. typhosus* cannot be recovered after the third day from soil contaminated by bacilluric urine; nor, after the fourth day, even from urine containing originally 60,000,000 *B. typhosus* per cubic centimetre.

On the other hand there are these factors: (i) The rains raise the subsoil water into direct communication with all urine pits and all wells in the station; (ii) Percolation can carry the *B. typhosus* through a 15 ft. belt of sand; (iii) *B. typhosus* and its allies are far more motile than *B. coli*, which frequently finds its way into filtered water supplies. Direct flow, percolation and motility may all combine to carry infective organisms from the pits towards the wells. In stations obtaining drinking water from a distant source cutting of the pipes at any time, by accident or by the enemy, would force large bodies of troops to drink water drawn from wells in subsoil levels saturated with percolating urine. He would be a bold man who would recommend this measure for general use while realizing that our knowledge of the transition and involution forms of specific organisms in their saprophytic phase still consists of a few scattered negations.

(*d*) *Evaporation.*—Three chemical firms having declined to make an offer for residual urinary solids obtained by evaporation, it is doubtful if this by-product can be worth anything approaching the sum stated; as, however, it is evident that its inorganic salts alone must possess a very definite manurial value, experiments have been persevered with. This question being the crux of the whole incineration problem, the time spent in briefly summarizing the results of those experiments will not be wasted.

Experiment 1.—The urine of a company (200 pints *per diem*) was all passed into a receptacle containing 90 lb. of sawdust, and having a surface exposed to permit evaporation. During the first four days, the season being the monsoon, the whole of it was evaporated. On the fifth day some effluent appeared, but this was inodorous. In order to delay the appearance of the effluent until the eighth day, and maintain it sterile indefinitely, the sawdust had to be increased to 270 lb. The sawdust at the end of the week contained some 13 per cent of its weight of urinary solids. The salinity of the urine in the upper layers was not, as was hoped would be the case, so great as to arrest

fermentation, and great complaints of smell were made. It is clear that flies, offence and cost of material make the adoption of this method impossible.

Experiment 2.—Charcoal was tried next, an improved V-shaped trough being made of layers of charcoal upheld and enclosed by wire netting. The dry season results were: 225 lbs. of charcoal were needed per company; 1 per cent of effluent appeared on the sixth day, being sterile, odourless and so saline as to be anti-bacterial; flies abounded and the ammoniacal smell from the upper layers was much complained of.

Experiment 3.—The apparatus used is diagrammatically shown in fig. 1. A 3 ft. cube frame was enclosed with wire gauze. Urine

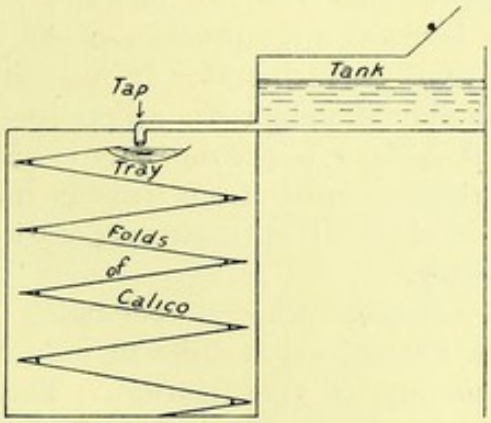


FIG. 1.—Urine Evaporator.

was conveyed by means of a tap into a shallow gutter in the roof, in which one end of a piece of calico, 60 ft. long by 3 ft. wide was fixed. The urine was sucked up by capillarity and flowed down the length of the calico, which zigzagged down the chamber, turning over horizontal wires at each side alternately. The flow of urine was adjusted by a tap so as to keep the whole of the calico moist. The total evaporating area (on both sides of the calico) was 360 sq. ft., and free access of sun and wind was secured, the folds being edge-on to the prevailing wind.

Theoretically the evaporation should be 15 gallons per hour at 85° F.; experimentally it was found to be 10 gallons, owing to lowering of the temperature by evaporation. This would appear to be most satisfactory, but strong complaints were made of ammoniacal smell, and it was found practically that the material sagged, so that urine dripped straight through the centre and the periphery

dried up, while the loose stuff threw off a fine spray as it flapped in the wind. The gauze kept flies away, but the spray was carried to a considerable distance, and this was an obvious danger. The little effluent that came through was sterile owing to excessive salinity caused by concentration.

Experiment 4.—Dishes containing 100 grm. of water, with 3 sq. in. of surface, were exposed for an hour in the hot-air sterilizer at a temperature of 80° C. The evaporation amounted to 22·6 grm., which could be increased 25 per cent by adding sawdust, and 37 per cent by adding charcoal to the water. The sawdust sank when saturated. The evaporation of water without the addition of charcoal or sawdust is equivalent to roughly 0·23 gallons per square foot per hour.

Having thus proved that 1 sq. ft. of water gives off 5½ gallons in the twenty-four hours at a temperature of 80° C., it was thought that the temperature of an incinerator furnace should reach 60° C., and at that heat it should induce an evaporation of 3 gallons per square foot of fluid surface per twenty-four hours. This raised hopes that evaporation of urine from trays in incinerator furnaces might be adopted in place of open-air evaporation, which had not been found practicable.

Experiment 5.—Details properly belonging to a subsequent section may here be anticipated in order to follow consecutively the development of this line of investigation. The evaporating tray adopted is shown in fig. 2. It forms a shallow receptacle fitted to form a false roof to the furnace—where the full heat is utilized by convection—and it is then extended to form the floor of the 3 ft. wide flue, where its water surface is exposed freely to the hot air which it was hoped thus to saturate with water-vapour. The furnace is connected with the flue by means of a funnel in the tray. The tray is fed with urine from a tank on the roof by a tap, which is automatically controlled by a ball-valve, so that the tray may neither overflow nor dry up and burn through. Provision is made for the tray to slide out for removal of the urinary solids deposited by evaporation. The following results were obtained :—

(i) With a tray of 9 sq. ft., evaporation never exceeded 2·5 gallons per square foot in the twenty-four hours.

(ii) On increasing the area to 17½ sq. ft. the total amount of fluid evaporated only increased by 3 gallons (from 23 to 26 gallons) in the twenty-four hours, while the rate per square foot per twenty-four hours fell to 1·44 gallons.

Disposal of only 26 gallons certainly did not justify continuance

of a device which added considerably to the cost of incineration and, although mechanically successful, was open to legitimate objection as being complicated and liable to get out of order.

(iii) It was further found that the evaporation was reduced to but 2 gallons per twenty-four hours if the fires were damped down by moistening the fuel and thus reducing the combustion rate, i.e., so much of the caloric value of the fuel was absorbed in evaporating the fluid from the saturated litter fuel in the furnace, that little remained when the air reached the urine in the tray; hot air left

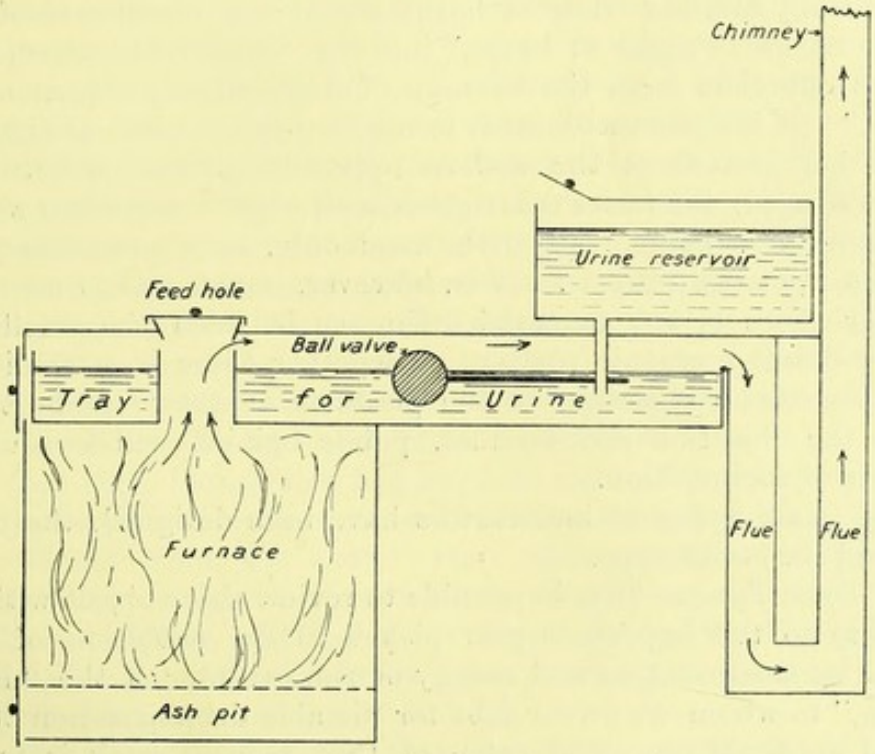


FIG. 2.—Incinerator with urine evaporating tray.

the furnace nearly saturated with water-vapour. This aspect will be more fully dealt with when we come to consider the physics of incinerators. It is sufficient here to point out that these experiments wholly contra-indicate the use of accessory evaporating devices in incinerator furnaces.

The conclusions drawn from this series of experiments may be thus summarized:—

(i) In the present state of our knowledge it is unjustifiable to dispose of urine by running it unsterilized into the subsoil of cantonments.

(ii) Maintenance of an organization for *separate* trenching of urine beyond cantonment limits is costly and difficult to safeguard.

(iii) Evaporation of urine in the open air cannot be conducted with economy and safety.

(iv) Evaporating trays in incinerator furnaces are complicated and unnecessary, considering that litter is capable of evaporating more than its own weight of liquid, if it be fully saturated before being used as fuel.

(2) *Offence.*

It needs but the whisper of "incineration" to convert the "delightfully familiar odour of burning peat—so reminiscent of the cotter's Saturday night at home," into the "intolerable stench and noxious effluvium from the burning of indescribably objectionable matter." It is some satisfaction to relate that one lady, anxious to launch her protest at the earliest opportunity, was unfortunate enough to apply the latter description with eager haste when a new incinerator was being tried with wood only, as a preliminary to commencing work proper! It is, however, immaterial to consider whether there is any essential difference between the smells of various burning organic matters or whether there is hygienic as well as æsthetic objection to the products of their combustion; unless the objection can be met, public opinion will crush the progress of incineration.

Two main types of incinerators have been designed, the open type and the closed type.

(*a*) *Open Types.*—It is impossible to review this subject without a tribute to the important part played in the evolution of this method by the initiative and energy of the inventor of the "Raitt pattern," to whom we owe a debt for his able demonstration of its general applicability. The value of that pioneer work is in no sense diminished by the fact that open types of incinerator must now be admitted to be unsuited for cantonment use. The best type of open incinerator is probably that adopted at Mhow, and it is sufficient to quote the opinion of the Principal Medical Officer 5th Division on that station, where this type was worked under the personal supervision of the inventor: "The pungent odour from the night-soil incinerators was an intolerable nuisance."

(*b*) *Closed Types.*—These marked a distinct advance, in that combustion was more complete and offensive products were more fully oxidized, becoming simple and innocuous bodies. Such patterns as Haines's, Young's, Hawes's and Cameron's still proved to be objectionable, as is shown by the fact that the chimneys had to

be heightened until they discharged some 20 ft. above ground level. Such a type is in use in my own station—a Sialkote with a 20 ft. high chimney—and I have to record that, since writing my first article, I have become aware of a steadily increasing and more influential volume of complaint against this type. The station extends for about two miles in the direction of the prevailing wind, and the leeward end is constantly under a heavy pall of smoke and never free from an acrid smell, which becomes acute on windless days and especially towards nightfall. This will have to be remedied or our incinerators will be closed down. It is fortunate that we have a device which has been proved to deal most effectually with this difficulty and which is as simple as it is effective. If the top of the chimney be splayed out into a chamber, which is plugged with a bundle of dried litter, the retarded current of air deposits the greater part of its smoke and some condensed steam upon this litter filter. In addition, there is a heavy deposit of tarry material, to which carbon particles adhere. The resulting extensive surface of water and carbon absorbs a large proportion of the foul gases evolved during combustion, as well as entangling the soot. The more the chimney is splayed out, and the greater the resultant retardation of the current through it, the more completely will the deposition and absorption be effected.

One such incinerator has been in action for two years within 30 yards of a barrack-room, without any complaint being made by, or elicited from, the men. One officer, admittedly opposed to cantonment incineration, was taken directly up the wind towards this installation and failed to detect its presence until within 50 yards or so, although it was then working at its usual pressure and the chimney was but 12 ft. high.

It is probable, however, that the offence complained of is caused less by the escape from the chimney than by that which results from the constant opening—and leaving open—of the lid by the sweeper when stoking and feeding the furnace. It is obviously preferable to empty each *gumlah* as soon as used rather than to adopt the objectionable alternative of storage in *balties;* the tendency of the sweeper to leave the lid open must be checked. This has been done by fitting the lid with a water-sealed rim and hinging it with a counter-balancing weight and a flange, so that it is opened by the foot of the sweeper to only a certain angle and slowly closes of its own weight as soon as his foot is removed from the lever. Dusting of *gumlahs* with lime is found to be most effective as a means of keeping flies away, and the covering of dejecta

with a layer of ash prevents offence while the *gumlah* is being carried from the latrine to the incinerator.

(3) *Fuel.*

This most important consideration controls not only the cost but the practicability of this method of conservancy. It will be best approached by the tabulation of certain essential data.

(*a*) *Amounts of Excreta, &c., to be Consumed.*—

Oz. Per Head in Twenty-four Hours.

	British troops		Native troops		Bazaar population
Fæcal solids	1	..	1½	..	1½
Fluid in fæces	3	..	6½	..	6½
Urine passed with fæces	5	..	5	..	5
Antiseptic fluid in *gumlahs*	10	..	—	..	—
Washing water	—	..	10	..	10
Urine collected separately	45	..	30	..	10
Ounces total fluid ..	63	..	51½	..	31½

Gals. per 1,000 Strength in Twenty-four Hours.

	British troops		Native troops		Bazaar population
Gumlah contents, without antiseptic ..	50	..	72	..	72
,, ,, with antiseptic* ..	112	..	—	..	—
Total, without *gumlah* antiseptic ..	331	..	322	..	199
,, with ,, ,, ..	394	..	—	..	—

Notes.

(i) Europeans pass daily fæces 4 oz., of which 75 per cent is water (3 oz.). Natives ass daily fæces 8 oz., of which 80 per cent is water (6½ oz.).

(ii) It is difficult to get natives to use urinals. The 30 oz. given above represents probably considerably more than the average per head collected daily from native units. The amount collected from bazaars was twice measured, it was respectively 7 per cent and 16 per cent of the total calculated to have been passed. This will cause no surprise to those conversant with native habits.

(iii) It is probable that lime is as effective as liq. cresyl. in keeping flies away from latrines; this is very important, as present regulations result in the addition of a considerable bulk of fluid to that which has to be disposed of.

(*b*) *Amounts of Fuel Available.*—(*i*) A cavalry regiment receives 2,400 lb. of bedding grass *per diem*, of which it is estimated that one half is eaten or stolen, thus leaving 1,200 lb. available. To this must be added the droppings, at 12 lb. per animal *per diem*, i.e., 4,800 lb., or a total of 6,000 lb. a day. Actual measurements for a month showed that 415 cubic ft. were collected on an average a day, which, at 15 lb. per cubic ft., gives 6,225 lb. a day.

(*ii*) I have no idea of the amount of sweepings available from

* Not used in native latrines.

dismounted units. Such rubbish is valued at one anna per cart, which, at 10 lb. per cubic ft. and 40 cubic ft. per cart, amounts to 400 lb., or say 300 lb.

(*iii*) A practically unlimited supply of grass is available; at grass-farm rates this costs 5 annas per 100 lb.

(*c*) *Amounts of Fuel Required.*—As fæcal solids provide for their own combustion, the matter is much simplified by the preceding table, which reduces the whole problem to the evaporation of so much fluid for each class.

All available data have been carefully collected. It is regretted that they are incomplete in most instances, but striking of averages for all comparable conditions has enabled many gaps to be filled. From the mass of results thus digested, there has been worked out the one essential datum, which is that showing how many pounds of fluid have been evaporated per pound of fuel expended.

(i) Open Patterns.

Station	Type	Lb. fluid per 1 lb. fuel. During monsoon	Dry season
Average of six ..	Raitt	Impracticable ..	0·80
Average of five ..	Hunt	0·25 ..	0·95
	(ii) Closed Patterns.		
Dehra	Young	— ..	0·75
Wellington	Wellington	— ..	0·72
Ambala	Ambala "B" ..	— ..	0·80
Landour	Landour (model) ..	— ..	1·00
Meerut	Lancer	1·23 ..	1·34

Notes.—(i) The Lancer pattern referred to above is one in which there is a urine evaporating tray in the furnace. These observations are now two years old. They were most carefully made daily from June 8 to November 20, and included exact weighings of fuel and measurements of fluid disposed of, through the monsoon and on into the dry season. The results obtained are so much better than those resulting from the use of other types that the following experiment was conducted to serve both as a control and to afford a comparison between the maximum evaporation from tray and furnace combined and that from the furnace only. Of the 1·23 lb. of fluid per 1 lb. fuel, 0·3 was evaporated from the tray and 0·93 from the furnace.

Experiment.—A foot-cube model was made of iron, of the type which will be described as our latest modification. It is evident that this small model could not be expected to give as good results as the incinerator itself, but the fuel and fluid were most carefully measured, and it was proved by five trials that the model would

easily deal with 1 lb. of liquid per 1 lb. of fuel consumed. Although there was no cause to doubt the accuracy of the Lancer pattern results—taken by three men over so long a period—it was satisfactory to have the added assurance of experiments conducted in the laboratory, and it was also satisfactory to find that the furnace alone was capable, even in this small model, of dealing with 82 per cent as much liquid as from tray and furnace combined.

(ii) Careful scrutiny of the figures will detect disparity between the ratio given and the claims advanced on behalf of the open types. This is explained by the recorded measurements of the amount of urine actually collected in bazaars, where these installations have mostly been used. Measurements have not been given, but it has been broadly stated that incinerators of certain dimensions have disposed of the total solid and fluid excreta of so many persons. The report has been quite true, but the urine collected and thus disposed of has, as shown, amounted to but a fraction of that passed, e.g., it is stated that a 7 ft. diameter Raitt will deal with the total excreta of 750 persons—i.e., 250 gallons, or 7 gallons per square foot. Experimentally it was found, however, that the maximum charge of fluid per square foot of surface, which did not drip right through within a couple of minutes, was 0·5 gallon, so that it would be necessary to have urine sprinkled on it at least fifteen times in the twenty-four hours, which could not be done, as the sweepers work only twelve hours a day.

We are now in a position, with the foregoing data, to state the actual fuel requirements as follows :—

Lb. of Litter or Grass Fuel Required Per 1,000 Strength Per Day.

	British troops	Native troops	Bazaar population
Gumlah contents, without antiseptic ..	400 ..	574 ..	574
,, ,, with ,, ..	896 ..	— ..	—
Total, without *gumlah* antiseptic ..	2,648 ..	2,560 ..	1,592
,, with ,, ,, ..	3,152 ..	— ..	—

For natives this provides for a considerable margin over the amount of fluid actually collected.

The factor used in this calculation is 1·23, viz., the amount of fluid evaporated by 1 lb. of fuel at Meerut during the monsoon. That this figure does not represent the possible maximum is shown by the fact that on July 18 the fluid evaporated per 1 lb. of fuel was 1·6, while on August 10 it rose to 1·7, but it should be noted that on those dates facilities were afforded for rough-drying the litter beneath an open shed.

It will further be noted that there is much less difference than would have been expected between the results obtained during the dry and wet seasons; this is probably accounted for by the fact that the relatively high temperatures at which this experimental incineration was conducted made the humidity of the external atmosphere of comparatively little consequence.

Conclusions.—(i) The total fluid and solid excreta can be disposed of by incineration with 2½ lb. of fuel per head per day.

(ii) Using this quantity of fuel, the litter of one cavalry regiment will dispose of the total excreta of 2,000 men.

(4) *Flies.*

With regard to flies, we wish to prevent their gaining access to human dejecta, and, as an extra precaution, to reduce their numbers.

All modern latrines now have an enclosed space for the receptacle, which is supposed to be fly-proof. Actually it seldom is so, and hence the following routine has been adopted: *Gumlahs* have a thick layer of ash placed in them, on which is dusted some lime; immediately after use it is the duty of the sweeper to cover the dejecta with ash and empty it forthwith into the furnace. The ash is sufficient to absorb all the dejecta, and the whole contents fall out in one mass, leaving the *gumlah* quite clean and dry so that it has no attraction for flies. Practically it is found that, although some flies may gather about the incinerator, attracted by its moisture, there are very few in latrines in which this routine is properly carried out.

The prevention of fly-breeding in litter is more difficult, but is surmountable with a little care, although the difficulties are enhanced in the rains when the litter must undergo some preliminary drying. All litter should be carried daily to the drying-shed beside the incinerator. This shed consists of a metal roof with fly-proof gauze sides and iron bars about a foot above the concrete floor. It has a feed-hole at the top of one end and a rake-hole at the floor level of the other end; both holes are protected by fly-proof doors closing by gravity. The litter is thus passed through the chamber, which should not be of a capacity of more than two days' supply. Some provision must be made for removal of the drainage which drips through the bars on to the floor; this is necessary not only on account of flies, but also to prevent it affording breeding grounds for mosquito larvæ. This is secured by raising the floor somewhat and giving it a slope, so that shallow

runnels lead the water to a pipe opening into a properly lidded receptacle which should be emptied daily.

(5) *Types.*

We now come to the most interesting part of this complex problem—the consideration of various types of incinerator. It is hoped that none of the many details hitherto dealt with has been irrelevant, and their consideration has, incidentally, disposed of many preliminaries to a review of the numerous types which have been described.

(*a*) *Open Types.*—It has already been shown that these types are not suitable for cantonment use owing to the offence to which they give rise. It has also been shown that they will not deal with the amount of fluid which it is essential to dispose of. It may be here pointed out also that they will not work in the rains unless covered, while they are sources of very considerable danger of dissemination of infective material by wind unless they are also enclosed by wind-screens. If they must be roofed and enclosed it is clear that they approximate to the closed type; but they cost more than the latter, while their combustion is less under control. In short, for use in British lines they are obsolete and discredited.

(*b*) *Closed Types.*—(i) Primitive patterns. These—of which Hawes's was the pioneer and the Sialkote is the modern representative—consist merely of a furnace in which the mixed fuel and excreta are burnt together and the fumes are led off by a chimney. The creation of a nuisance has not been obviated by the heightening of the chimney, although that now discharges 20 ft. above the ground level (*vide supra*). Various attempts have been made to destroy these fumes, and the suggested devices may be divided into two groups, according as the dejecta are burnt with, or in a separate chamber from, the fuel.

(1) *Dejecta in a Separate Boiler.—Thompson's (Quetta)*: This is shown in fig. 3 (a) (i). The fumes pass from the boiler down a central pipe and so escape through the fire, which is supposed to oxidize them. At first it was found that the contents frothed over and put the fire out. This was prevented by the addition of a perforated diaphragm (shown dotted), which broke up the froth and allowed the fluid to run down its upper surface into the boiler again. It was found that it took over twenty-four hours to burn out and was costly in fuel.

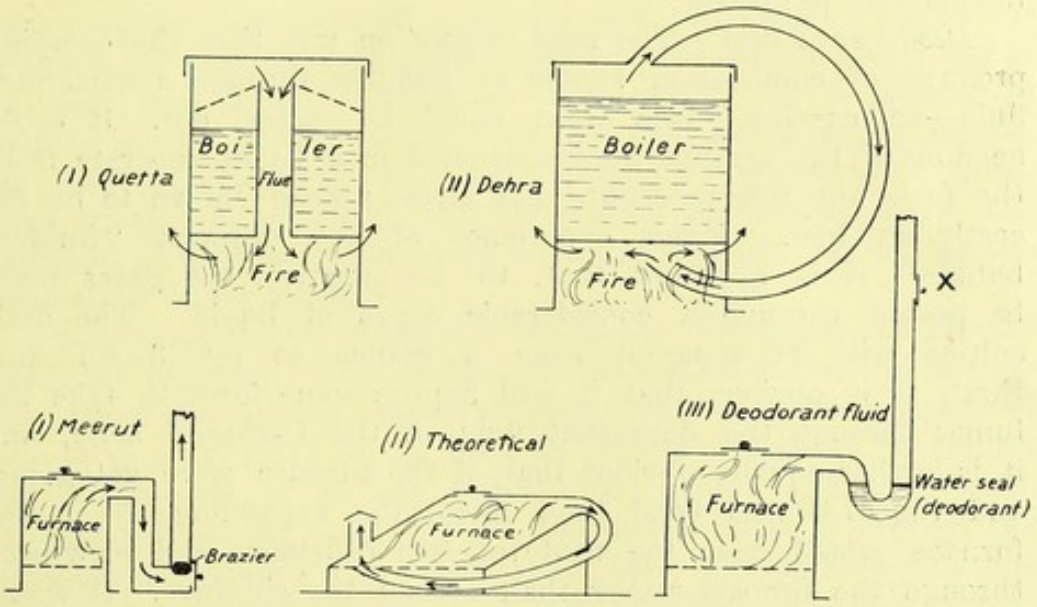
Dehra Dun, shown in fig. 3 (a) (ii): This is somewhat similar, and it "took sixteen hours to incinerate, with excessive fuel consumption." It is evident that the combustion of excreta and fuel in

separate chambers must involve enormous waste of the latter, and this idea was therefore not pursued further.

(2) *Dejecta and Fuel Mixed.*—Attempts were made to pass the fumes through a secondary fire, through a part of the main fire, through a deodorant fluid, and through litter used as a filter.

Meerut.—The flue was taken down to ground level, where it was splayed out to permit of deposit of heavy soot particles. The flue then narrowed to the chimney foot, where a small brazier of

(*a*) With a separate boiler for fluids.



(*b*) Fuel and dejecta mixed in the furnace.

FIG. 3.

charcoal was inserted. It was hoped that the amount of carbon monoxide present would prevent rapid combustion of the charcoal and maintain it economically at a dull red heat, which would destroy the fumes passing through. Also, by thus heating the air in the chimney, it was hoped to secure extra forced draught through the furnace. Practically this device served its purpose, but it burnt out in a few minutes and proved too costly for use. It is shown in fig. 3 (b) (i).

Theoretical.—Shown in fig. 3 (b) (ii). It was proposed to set the furnace at an angle, so that the red-hot layer would slide down into one corner. The fumes were to be brought by a pipe which discharged beneath that corner, so that they passed up

through it, to escape by a small chimney immediately above. This escape chimney being below the main body of the fire, it was thought that no fumes would escape directly by that route. Unfortunately there were no means of preventing chokage of the end of the pipe by ashes falling directly into its mouth; moreover, there would obviously be great difficulty in establishing and maintaining a through draught, therefore the idea was abandoned as impracticable. At this time it was realized that, if any such simple device were feasible, it would long since have been taken advantage of for commercial purposes, and not left to would-be devisers of incinerators to evolve.

Deodorant Fluid.—The next suggestion was that the escaping products of combustion should be bubbled through a deodorant fluid—an excellent idea if it could be carried out. It could be done if the excreta were consumed in a boiler separate from the fuel, but this procedure has already been shown to be too costly in fuel. From experience of the ordinary "hubble-bubble," it is apparent that, to be effective, the gases must be passed through a considerable depth of liquid. The difficulties will be apparent from a glance at fig. 3 (b) (iii). First, it is obvious that it will require some force to take the fumes through the deodorant fluid in the U-shaped bend, and it is further equally obvious that, if the furnace were got going, and the U-shaped bend then filled, the expanding air in the furnace would take the route of less resistance and blow back through the firebars rather than ascend the chimney. It would therefore be necessary to secure a suction effect by a secondary fire near the top of the chimney—as indicated at "X"—which would involve all the expense indicated under that proposal initially. Further, some device would have to be adopted by which the supply of fluid in the bend would be renewed automatically; and there is the great objection that the sweeper would have to be responsible that the fluid was renewed when the deodorant was exhausted; while, finally, as an additional complication, the supply of deodorant would have to be maintained.

Litter Filter.—This has already been discussed. The design is shown in fig. 4. It has the advantages of being so simple as to be within the scope of even the sweeper's intelligence, of requiring only material always at hand, of costing nothing, and being renewable in a few seconds, of being itself combustible, owing to the condensation of the products of partial combustion in its interstices, and, finally, of being of proved efficiency. It can be inserted at

any point in the ascending flue, the only essentials being that the sides of the chimney beyond it should be air-tight, and that the door through which it is inserted should fit so closely as to prevent short-circuiting of the forced draught produced by ascent of heated air through the chimney. Its efficiency becomes greater as the tarry deposit increases, it is advisable that two such filters should be used in the circuit, to be renewed alternately on alternate days, or as may be found necessary.

Patterns Fitted with Devices for Aiding Evaporation.

In the simplest of these designs a receptacle from which urine was evaporated was placed in the furnace. The smell of urine increased the offence considerably without an adequate return, for it is clear that a tank affords so little surface in proportion to bulk as to make the amount of urine thus disposed of inconsiderable. In other patterns the urine, after it had been supposed to be boiled, was drawn off by a tap, and thrown on the surface soil. The decision as to whether the urine were sterile or not was left to the discretion of the sweeper!

Next came the era of trays, of which the Wellington and Meerut were the pioneers. That at Meerut has been described—it has the advantage that urine is evaporated completely, while the exposure of the large area of 17½ sq. ft. for 6 in. of depth enabled 26 gallons to be disposed of in the twenty-four hours. Difficulty arose, however, from the need of some automatic device to prevent the tray being overfilled, thus flooding the fire. For the first time this installation enabled the whole excreta, fluid and solid, of a unit to be disposed of with a moderate consumption of fuel.

This was followed by a most ingenious invention at Ambala. There the urine was placed in cylinders in the furnace. The cylinders had a number of small perforations in the base, which were covered with a layer of 1½ in. of wood ash. When the urine boiled, the ebullition allowed the urine to drip through the ash on to secondary evaporation trays placed beneath. There are, however, two very strong criticisms to be offered to this installation: (*a*) It is clear that, if the urine is to be boiled in these cylinders, there must be fierce combustion in the furnace, and a large proportion of the caloric value of the fuel must be wasted as the heat passes the cylinders to the chimney. This is evidenced by the fact that the fuel required in this pattern is 60 per cent greater than that of the Lancer pattern. It would appear that this ingenious idea might be turned to better advantage if it were applied to a shallow tray of large area, so as to secure a uniform distribution of urine upon the

mass of litter in the body of the furnace. It is a mistake to suppose that leading the fumes from the cylinders into the chimney has disposed of the offence which is inseparable from the evaporation of urine. A certain amount will doubtless be absorbed by the soot in the chimney, but that will be but a small fraction of the whole. Without some means of dealing with the nuisance, this installation is just as offensive as the Sialkote type, and it has been shown that strenuous objections are being made to the latter.

Suggested pattern for Landour. It is advisable to pause here and, in the light of the practical experience gained, consider the physics of the means whereby we hope to attain our triple aim, of (α) maximum evaporation, (β) minimum offence, and (γ) minimum fuel expense.

The caloric value of the fuel consumed in incineration is expended on :—

(1) Evaporation of fluid.

(2) Maintenance of the draught necessary for continued combustion.

(3) Loss through the chimney of heat in excess of that needed for draught.

(4) Loss of heat by radiation, &c., from the exterior.

Now (4) is probably a constant, whatever our rate of combustion, and not worth the cost of prevention by building a fuel chamber around the furnace. (3) should be capable of reduction to small dimensions by practical application of sound principles. (2) is a matter of friction and is intimately concerned with the removal of nuisance caused by the smoke and fumes. If this can be assured, the extra expense of fuel incurred thereby is well spent. (1) is the most important hygienic consideration, as upon it depends our ability to dispose safely of urine.

To return to our aims—

(α) *Maximum evaporation*.—The ideal method is that which secures an intimate admixture of excreta with a "matrix" fuel, which provides the fluid with an enormous evaporating surface in proportion to its bulk, forms a spongy mass, through the interstices of which hot air percolates until saturated with water vapour, and automatically ensures that the fuel cannot burn until its charge of moisture has been evaporated. If the liquid be separated from the fuel by enclosure in a boiler, the furnace heat only transmits a part of its value to the boiler contents, while the remainder roars to waste up the chimney. The importance of this point is evidenced

by the fact that, as shown, the matrix-fuel method requires but 62 per cent of the fuel needed by the alternative boiler method.

It was hoped to ascertain, by the model referred to, whether slow or rapid combustion gave the better result—the rate of combustion being controlled by differential temperature readings from thermometers in the furnace interior and in the external air. The records have unfortunately been lost, but it is highly probable that slow combustion is the better, and, if so, this affords an additional argument in favour of the matrix-fuel method, which works continuously throughout the twenty-four hours.

(*β*) *Minimum of Offence.*—This raises some interesting points for consideration. The only practicable and effective means evolved so far is that of the litter filter, the efficiency of which depends upon its extent of surface, the moisture of that surface, the retardation of the current through it, and the filter being left long enough to be coated with a carbonaceous tarry deposit. Extra fuel must be used to overcome the additional friction thus thrown into the circuit, though this may be lessened by heightening the chimney.

It is found experimentally that, if the caloric value of the fuel be utilized to within 5 per cent of saturation point by water vapour, the remaining energy is sufficient to afford the necessary balance for meeting friction and loss. At that point water is progressively deposited in the litter filter if that be placed in an external chimney. It is obvious that the litter filter must be placed in the ascending chimney so that the ascent of the hot air on the distal side of the filter secures aspiration and forced draught sufficient to maintain combustion. The objection to this is that a certain amount of water condenses continuously and either has to be evaporated afresh or else causes nuisance by soaking through the chimney and running on to the ground. Also the cooling of the hot air in the interior of the chimney throws extra work on the furnace, and might just be sufficient to determine the equilibrium point at which the fire would be put out if it were too much damped down at night.

It is proposed to meet both these difficulties by taking the flue through the heart of the furnace, before it turns to ascend through a fuel drying chamber on the furnace roof. The litter filter would then be inserted in a box on the portion of the flue inside the furnace, the box having an iron door in the furnace wall. By this means water would condense at once on the filter, but, once deposited, would be maintained at that amount by the

balancing forces of condensation and evaporation—with the cooling action of radiation from the door just turning the balance. Moreover, the temperature inside the flue would adjust itself to that of the furnace and energy would thus be economized, with a consequent lessening of any tendency for the fire to go out. It is probable that this is not actually necessary, for the Lancer pattern described worked night and day for twelve months, during which time it only went out accidentally twice.

(γ) *Minimum Fuel Expense.*—This has been sufficiently discussed under the two preceding headings.

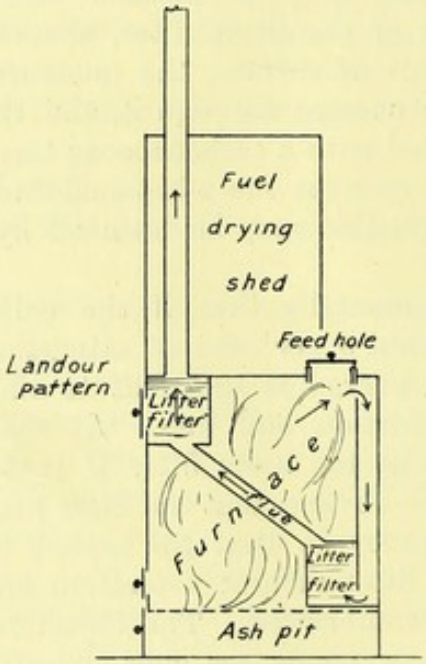


FIG. 4.

Conclusions.—(i) That the admixture of excreta with matrix fuel and combustion in a furnace affords the maximum evaporation of which the caloric value of the fuel is capable.

(ii) That this simple method obviates the necessity for any accessory means to evaporate fluids.

(iii) That offence can be obviated to a large extent by the use of litter filters at a small increase to fuel expense.

(iv) That such litter filters are best situated in a portion of the flue taken through the furnace for that purpose and for the more certain maintenance of combustion.

It only remains to remark that the drawing of this type, now in course of erection, appears in fig. 4 and that its soundness of principle has been thoroughly demonstrated by experiment with a 1 ft. cube iron model, which has disposed of 1·25 lb. of water for each 1 lb. of fuel consumed, and when once heated, burns quite steadily without extra forced draught.

(*B*) Cost of Conservancy if Incineration is Adopted.

This section, fortunately, can be dismissed in a few words. It has to be considered from two standpoints, according as the value of the litter to the grass-farm authorities is, or is not, taken into account.

(1) *Not considering the value of litter to the grass-farm.* — It has been estimated that, at Meerut, the cost of conservancy to one British cavalry regiment has been Rs. 373 per 1,000 strength per annum less since incineration was adopted.

At Dehra Dun it has been calculated that the cost per annum per 1,000 strength of native infantry has been reduced by Rs. 216 per annum.

At Ambala it is stated that the comparative costs are "about the same" for the conservancy of British troops by the two methods.

At Meerut it is estimated that the establishment of forty-seven incinerators in cantonments has resulted in an economy of Rs. 4,000 per annum, the population being approximately 12,000, this amounts to a saving of Rs. 350 per 1,000 strength per annum.

These illustrations suffice to show that incineration enables a definite economy to be effected.

(2) *If the value of the litter to the grass-farm be considered.*—It has been seen that the litter from one British cavalry regiment amounts to 6,000 lb. per diem. The grass-farm authorities claim that this litter enables them to surface-trench six acres per annum, with an increase in the grass crop from 100 to 600 maunds per annum for eight years. Before this estimate can be accepted, information is required showing how much of this increased crop is due to turning over the soil, and how much to the manurial value of the litter. Until that information is forthcoming it is impossible to say what the net cost of incineration really is.

(*C*) Effect of Adoption of Incineration upon Military Efficiency.

With so many improvements in the hygienic conditions of the troops in India, and marked advances in the art of preventive

medicine, it is impossible to allot to any one measure its share of credit for the remarkable diminution of sickness amongst the troops in India which has characterized the last few years. It is, however, significant to note the unanimity with which those most directly in touch with the advances and shortcomings of Indian sanitation—the divisional sanitary officers—attribute, in their annual reports, a large share in the diminution of sickness in their respective divisions to the more general adoption of incineration as a means of disposing of dejecta. It may well be that only on general principles are we entitled to that belief, but none the less is that belief in its turn entitled to credence if based on the application to conservancy methods of definite knowledge of specific disease acquired by the accumulated experience of many years of patient research. This much at least is certain—the sanitation of native units more especially has left, and still leaves, much to be desired, and the attention that has been drawn to sanitary conditions (or perhaps one should rather say to *insanitary* conditions) by the introduction of incineration has already had a most beneficial effect in its educational influence upon the attitude towards sanitary matters adopted by both officers and men.

———

QUININE AS A MALARIAL PROPHYLACTIC: A CRITICISM.

By Captain P. S. LELEAN.
Royal Army Medical Corps.

There are few questions of tropical preventive medicine of more practical importance than that of the value of quinine in malarial prophylaxis—an importance enhanced by recent advocacy of a policy of reliance upon this method of prevention, to the exclusion or diminution of efforts directed towards reducing mosquito prevalence. That policy appears in India to be due largely to a somewhat enthusiastic representation of the success of quinine prophylaxis in reducing malarial incidence upon certain jail populations. One such representation has been circulated by the civil authorities in pamphlet form, and contains this summary of the views of its responsible medical author: "Take quinine regularly and one is absolutely malaria-proof; neither mosquito campaigns nor mosquito-nets, nor any other wonderful contrivance or device to deal with these insects is required."

Such a dictum on an important and complex problem would be comparatively harmless if presented only to professional men in a position to gauge its value accurately; it must, however, be considered seriously when cited by a non-expert administration as justifying the adoption of a policy so ineffective as to be ultimately as costly as it is initially cheap. The unfortunate consequences of this professional hemianopia were prominently brought to our notice in connection with an appeal for civil co-operation in dealing with the notorious unhealthiness of a military station, garrisoned solely on civil considerations. The reply to that appeal consisted of an expression of the pleasure felt in bringing to the notice of the military medical authorities a means of malarial prevention so simple as to render all other measures unnecessary—as shown by an enclosed copy of the pamphlet referred to above.

A study was at once made to see whether the available data afforded any support to these extreme views. The observations then made were so strikingly and unexpectedly adverse to the claims put forward that they have been revised and extended. The conclusions drawn from them have confirmed the initial observations to an extent which leads to the hope that their publication may prove interesting to those striving to combat what—in the

light of these data—cannot but be regarded as a policy likely to prove disastrous to the health of the Army in India. The conclusions drawn may be thus impartially summarized in the words of the Senior Medical Officer of the station referred to above: "I am greatly disappointed to find that my experience here does not support my previous ideas about quinine prophylaxis. Everything was done here last season to give it a chance, and yet I must admit that it has done little good."

These data may be dealt with under the headings of jail and military statistics in India, per mille rates being adopted throughout, and the latest figures obtainable from official returns being embodied. The jail rates are those for India and Burmah.

Jail Statistics for India and Burmah.

A.—*Analysis of the General Admission, Death, and Case Mortality Rates.*

Confidently postulating able medical administration, it was expected that the prophylactic use of a drug capable of rendering prisoners "absolutely malaria-proof" should afford striking evidence of its value when the above rates were charted. As the value of the method was demonstrated and more widely recognized, its more general adoption should have been reflected by a progressively rapid decline in the malarial admission-rate. The charts are reproduced, and it remains to consider to what extent they represent the actual prevalence of malaria and the influence of prophylactic quinine upon that prevalence. (Chart 1.)

If statistics affecting large numbers and extending over a series of years show, when charted, an unmistakable and well-marked general tendency, it is permissible for purposes of demonstration to bridge by a dotted line isolated years which break the uniformity of that general tendency. The malarial admission-rate of a jail population averaging over 97,000 shows during the past twenty years a general decline. That general decline is broken by two rises which, while larger than the actuarial limit of statistical error, are yet within the limits of seasonal variation. Comparison with the total-mortality and case-mortality rates makes it evident that 1901 and 1905 are the abnormal years; they are therefore bridged and the three curves, thus modified for facility of comparison, are projected to scale upon the same chart thus (Chart 2):—

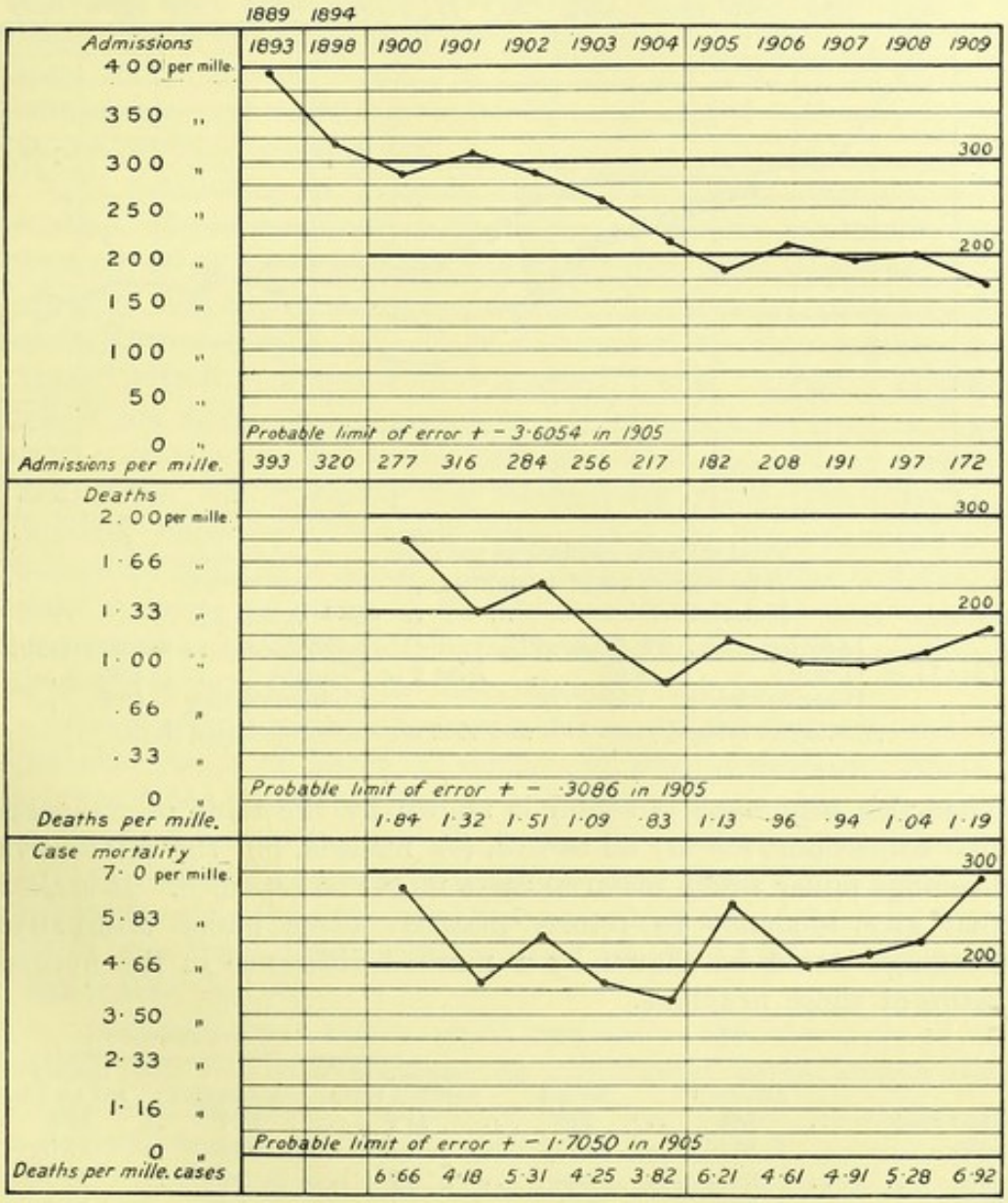
To what extent do these figures indicate the actual prevalence and severity of malaria?—Three essential factors are indicated by

the Sanitary Commissioner as influencing the value of malarial statistics of the jails. The following attempt is made to gauge their value.

(*a*) "*Great Divergence in Diagnostic Practice.*"

CHART 1.—*Indian Jail Statistics.*

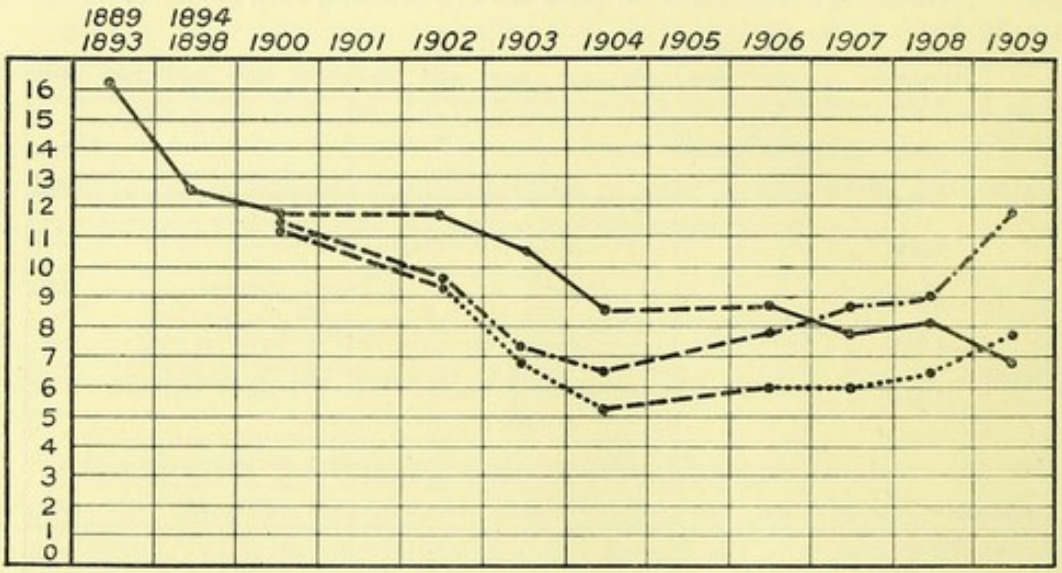
General malarial admission, death and case-mortality rate, per mille.



There is little room for doubt that this factor tends constantly to augment the malarial returns. In considering the malarial admissions of a series of years, it is evident that their relation to

each other is therefore only affected by variations in the degree of that augmentation. With so large and scattered a population and so many workers, variations in diagnostic practice should tend to equalize each other and balance out in the annual returns.

CHART 2.



Total malarial admissions per mille ——————
Total malarial case mortality ,, —·—·—·—
Malarial deaths ... ,, cases .....

Line 12 = { Admissions .. 300·00 per mille; Deaths .. 2·00 ,,; Case-mortality 7·00 ,,

Line 8 = { Admissions .. 200·00 per mille; Deaths .. 1·33 ,,; Case-mortality 4·66 ,,

N.B.—The bridged years 1901 and 1905 are shown by dotted lines.

That this argument is sound is shown by the table of comparative admissions, for (*a*) all causes, (*b*) malaria, (*c*) the alternative headings under which malarial cases might be returned. It is clear that any tendency to return malarial cases under alternative headings would be shown by a marked difference in the normal ratios of those headings.

	(*a*)	(*b*)	(*c*) Alternative Headings		
	All causes	Malaria	Pyrexia of uncertain origin	Influenza	Debility and anæmia
1900-1904 ..	**781** ..	**270** ..	**11·7** ..	**12·4** ..	**14·6**
1905	661 ..	182 ..	9·7 ..	11·5 ..	11·0
1906	620 ..	208 ..	9·7 ..	5·9 ..	12·5
1907	521 ..	191 ..	5·1 ..	4·9 ..	11·2
1908	656 ..	197 ..	9·4 ..	4·2 ..	11·8
1909	635 ..	172 ..	10·8 ..	1·5 ..	11·8
Average 1905-1909 ..	**618** ..	**190** ..	**8·9** ..	**5·6** ..	**11·6**

The percentage reductions which have occurred in the figures of the two quinquennia work out thus :—

Admissions from all causes	..	..	..	reduced to	79·1	per cent.
,, for malaria	..	..	..	,,	70·4	,,
,, of three alternative headings			..	,,	67·4	,,

It is evident that the entries of the combined alternative headings have fallen more rapidly than the malarial rate itself, so that there is no question of their having been inflated. Moreover their total combined figure is but 13·2 per cent of the malarial admissions, and variations must be enormous if sufficient to alter appreciably the malarial rate.

(*b*) "*Ascribing to Malaria Deaths due to some other Cause.*"—Malaria affords the simplest diagnosis, and easiest treatment, for a number of grave conditions associated with fever—whether intermittent, remittent or hyper-pyrexial. Conversely, relatively few malarial deaths appear likely to be ascribed to other causes—such being certain rare forms of "cerebral" and "algide" varieties, which are themselves uncommon. Here again we have a factor with a constantly augmenting influence upon the malaria statistics and hence one of which the fluctuations, rather than the total amount, need be considered with regard to the relationship of individual years of a series. This is supported by the fact that in some districts as many as 25 per cent of cases of post mortem examinations on deaths attributed to malaria showed the true cause of death to have been other than malaria.

Even after making allowances for errors of diagnosis and the probable limits of statistical error, and after considering Chart 1 as unmodified, there appears no reasonable doubt that the decline in the malaria death-rates which characterized the quinquennium 1900 to 1904 has not been maintained since 1904. This applies in greater force to the case-mortality rate which declined from 1900 to 1904; rose from 1904 to 1909; and stood in 1909 a few points higher than in 1900.

Statistical logic teems with intricacies and the path of the statistician with pitfalls, but the conclusions to be drawn from Chart 2—if its general accuracy be admitted—are so interesting and apparently conclusive that the path must be followed.

(1) If the total and case-mortality rates both rise and the latter the more steeply—as shown in Chart 2—it can be mathematically shown that this feature can only be produced by the association of two factors acting together. Those factors are (i) progressive diminution in the malarial admission-rate and (ii) progressive

increase in the relative tendency to a fatal ending of the malarial cases admitted.

These factors need consideration in the inverse order.

Factor (ii.)—*Progressive Increase in Mortality among Cases admitted for Malaria.*—Assuming that treatment has not become less skilful during the last decade, there remain two possible explanations of such an increase :—

(1) That quinine has lost its curative efficacy. This, in turn, must be due to :—

(*a*) A larger proportion of quinine-resisting, malignant infections among the admissions, which, apart from alteration of the normal ratio by elimination of mild cases from the returns, there is no reason whatever to believe has been the case, or—

(*b*) Increased resistance of malarial plasmodia to the curative action of quinine, of which the reasonable explanation is that constant use of ineffective "prophylactic" quinine has produced strains of plasmodia habituated to, and thus resisting, that drug.

(2) That mild cases have been eliminated from the returns, thus artificially increasing the proportion of malignant, resistant infections among the admissions.

To recapitulate—either prophylactic quinine has actually increased the number of deaths from malaria (by producing quinine-resisting strains), or else mild cases have been excluded from returns. The former alternative can hardly be acceptable to the advocates of this method; it remains to see whether the latter proves so. This brings us to :—

Factor (i.)—*Progressive Diminution in the Malaria Admission-rate.*—This may be real or artificial. It has just been shown that, if the mortality rates be approximately correct, a real diminution in the admission-rate must have been associated with an increase in severity, which, in turn, proves unacceptable. The only alternative is that the diminution must be artificial, and the result of omission of cases from statistics. It has also been shown that malarial cases have not been returned under alternative headings; they must, therefore, have been treated as out-patients and omitted from returns altogether. This constitutes the third consideration raised by the Sanitary Commissioner :—

"(*c*) *Treating Cases of Malaria without admitting them to Hospital.*"

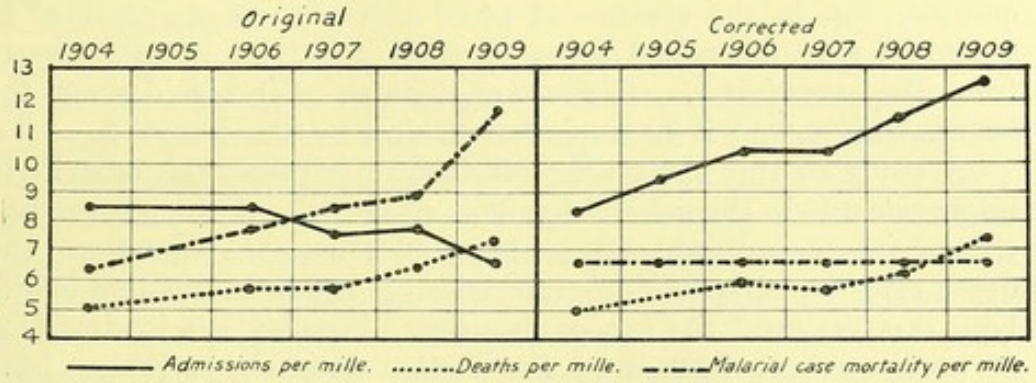
It is evident that admission (and return) of only relatively severe cases must proportionately raise the case-mortality, while having but little effect, *per se*, on the total malarial death-rate, which is

so largely maintained by those malignant forms on which quinine admittedly has slight action. It follows that the disparity between the total and case-mortality rates thus affords a gauge of the extent to which it has been the practice to exclude slight cases of malaria from the returns. The formula by which our chart may be thus corrected is simple :—

$$\text{Malarial admissions per mille} = \left\{\frac{\text{Total malarial deaths per mille} \times 1{,}000.}{\text{Per mille mortality of malarial admissions.}}\right.$$

It having been shown that the rise in case-mortality seen in Chart 2 cannot be accepted, it is instructive to re-chart the last quinquennium as it would appear without that rise.

CHART 3.



It is seen that, in place of a reduction, there has actually been a considerable increase in the prevalence of malaria during the past quinquennium. This alternative, therefore, would not appear to be any more satisfactory than the former, and the findings on either count are adverse to the contention that administration of quinine has rendered the jail population of India " malaria-proof."

Even if the malaria case-mortality rate be regarded as having remained constant during this quinquennium, the fall in malarial admissions shown by Chart 2 could only be associated with a 45 per cent error in the returns of malarial deaths, which cannot be regarded as possible. So much for the last quinquennium—what of that of 1900-1904 ? It is apparent that the decline in this period is an actual decline. It does not, however, necessarily follow that it is the result of prophylactic quinine, in fact consideration shows that the evidence points to the contrary. The argument is a brief one :—

(i) Quinine has least action on malignant infections, to which are due the greater proportions of deaths.

(ii) Therefore the results, if due to quinine, must show a greater

fall in admissions than in deaths, associated with a rise in case-mortality. The chart shows precisely the contrary in each respect; the fall in deaths exceeds that in admissions, while the case-mortality rate has fallen also.

In concluding this section, it is as well to refer to the general experience of clinicians with regard to the relative inefficacy of quinine in malignant infection, as diagnosed microscopically. This relative inefficacy is referred to by Castellani and Chalmers.[1] It is therefore obvious that the naked merozoites of malignant plasmodiæ must be more resistant to curative quinine than the plasmodia of the benign infections. It would be most difficult to prove that the same holds good with regard to the resistance to prophylactic quinine, but, in the absence of proof absolute, we are justified in assuming that the naked sporozoites do not differ in this respect from the merozoites. If curative quinine will not destroy the latter, why should it be supposed to destroy the former?

A study of this chart suggests that its explanation must lie in improvements in the general hygienic conditions of the jails, reduction of the mosquito-breeding places affecting the admission- and death-rates equally, while better housing and food further reduce both the total and the case-mortalities. Possibly the introduction of pure quinine, instead of the crude material, may also have assisted treatment and favourably influenced both mortality rates.

B. *Sidelights from the Statistics of Individual Jails.*

(a) Instances in which Prophylactic Quinine has been experimentally tried.

(i) *Peshawar and Dera Ismail Khan.*—These malarial stations were selected for comparison of the malarial incidence upon groups of prisoners. The groups were of equal numerical strength and average physique. Prophylactic quinine was administered to them in definite amounts, one group having all quinine withheld as a control.

	Year	Men	Quinine given	Malarial admissions. Total
Peshawar	1908	53	*Nil*	25
		53	5 gr. daily	49
		53	15 gr. on two days per week	53
	1909	30	*Nil*	11
		30	5 gr. daily	6
		30	15 gr. on two days per week	1
Dera Ismail	1909	47	*Nil*	26
		47	5 gr. daily	7
		47	15 gr. on two days per week	2

[1] "Manual of Tropical Medicine."

If the three experiments be summarized, the groups of 130 men each give malarial admissions per mille for the period of the experiment as follows:—

	Total	Per mille
(*a*) Getting no quinine..	62	476
(*b*) Getting 5 gr. daily	62	476
(*c*) Getting 15 gr. twice a week ..	56	430

It may be assumed that, in view of this being an experiment, there was no doubt but that the prisoners actually took their quinine and that it was given in an assimilable form. In spite of this the reduction in the amount of malaria achieved by this means was less than 5 per cent, while in the first experiment—made in a bad malarial year—the adoption of quinine prophylaxis doubled the malarial admissions.

Possibly the term "absolute immunity" is sufficiently elastic to cover admissions up to 50 per cent of the strength per annum. In any case, however, the Inspector-General of Jails attributes the "relative immunity" to malaria of Peshawar prisoners to other factors than quinine prophylaxis, to wit—"sleeping in airy barracks which afford no cover for mosquitoes," whereas the police, in adjacent quarters swarming with mosquitoes, give four times the malarial admission-rate of the prisoners. It is clear that the Inspector-General of Jails holds no brief for quinine prophylaxis.

(ii) *Gorakhpur and Saharanpur.*—"Two usually very unhealthy jails" were selected for a special experiment in prophylactic quinine in 1908. It is interesting to compare the total and malaria admission rates since 1907.

		Malarial admissions	Admissions for all other causes
Saharanpur	1907	516	1,015
	1908	336	856
	1909	238	638
	Reduction since 1907 by	47 per cent ..	63 per cent
Gorakhpur	1907	227	945
	1908	233	770
	1909	167	498
	Reduction since 1907 by	26 per cent ..	47 per cent

It is evident that the general hygiene has improved in these jails to such an extent that the total admissions from all other causes than malaria have diminished more rapidly than those for malaria—in Saharanpur to the extent of 16 per cent and in Gorakhpur to the extent of 21 per cent.

There appears, therefore, to be no more logical reason for

denying the influence of improved hygiene upon the malarial rates than there is for attributing to quinine prophylaxis the greater diminution in the admissions from all other causes combined.

(*b*) *Instances in which Prophylactic Quinine has not been used.*—The most striking example of diminution in malaria, which, to whatever other cause it may be actually due, is certainly not due to the effects of prophylactic quinine, is that afforded by the Lucknow jails and the Central Lucknow Jail in particular.

PER MILLE ADMISSION RATES.

	All Indian Jails		Lucknow				Jails of Group IV	
			Central		District			
	Malaria	All causes	Malaria	All causes	Malaria	All causes	Malaria	All causes
1907	191·3 ..	521	27·0 ..	165	72·3 ..	355	..	
1908	197·7 ..	656	8·3 ..	140	46·5 ..	328	251·5 ..	957

It is seen that, in 1908—the most malarial season for many years past—the Lucknow Central Jail gave an amount of malaria which was but 4·2 per cent of that of the rest of India and only 3·3 per cent that of the jails of the group to which Lucknow belongs, while the Sanitary Commissioner remarks " in this year (1908) Lucknow district and town suffered severely from malaria," so that the local conditions were unfavourable. Certain possible explanations were considered :—

(i) This is not a snap figure from a small jail—the 1907 rate was also a very low one, while the annual average strength in 1908 was 1,735.

(ii) Nor is it due to statistical dexterity—the total admissions fell by 15 per cent from 1907, they were but 21 per cent of the figure afforded by the convicts of India in 1908 and there was no case returned as pyrexia of uncertain origin in either year.

There was, therefore, a genuine and quite remarkable freedom from malaria in a most malarial year and in a malaria-infested district. To what was that freedom due? " The jail was three miles from the city and the superintendent attributes the exemption from malaria to the general excellence of the hygienic conditions. . . . . No special anti-malaria measures appear to have been adopted and there was no prophylactic issue of quinine."

It is interesting to make a comparison between this dictum on an 8·3 admission-rate and that on a 204·8 admission-rate, i.e., over twenty-four times as high : " I can state without hesitation that the source of immunity, and practically the only source, was the dosage of every prisoner with quinine 15 gr. per week." One is tempted to ask—what immunity? If a rate of 234 per mille is the expression

of being absolutely malaria-proof, one also wonders what figure would represent malaria liability.

If the Lucknow relative immunity were not the result of general sound hygiene, then it was the result of coincident factors of which we know nothing, but which are possibly dominant factors. It is clear that mere opinions are worthless and scientific investigation of facts is essential. As the Sanitary Officer of the Pindi Division condenses the matter: "For some years, indeed, investigatory work is more needed than any other form of energy."

(Since writing the above a communication has brought welcome indication that this view is also held at headquarters.)

(*c*) *Some Apposite Figures from the City Jail of the Particular Military Station referred to above.*

While quinine prophylaxis was being brought to our notice, and every sanitary canon was being meanwhile outraged by the civil population in the immediate vicinity of the garrison, the malaria admission-rate in the city jail was steadily rising, 18 per cent in two years.

1907	Malaria admissions	per	mille	= 245
1908	,,	,,	,,	= 285·1
1909	,,	,,	,,	= 295·5

An interesting light is thrown on these figures by the most instructive observation—made by no less an authority than the Director of the Malarial Research Laboratory—that, despite prophylactic quinine, of the prisoners in this jail in the autumn of 1909, the blood of 37 per cent of those examined contained malarial parasites.

Indian Military Statistics.

It is a relief to step from the shifting morass of statistical argument on to the terra firma of established facts, which leads to the explanation that, but for this solid take-off, the plunge into the morass would never have been essayed.

The following tabulated information was obtained from stations in the division in which at least 20 per cent of malarial diagnoses had been confirmed by the microscope. Of the whole series, 64 per cent of bloods were examined, and of these 62 per cent confirmed the diagnosis, i.e., 39·7 per cent of the malaria admissions were proved to be malarial. That figure, however, must not be taken as the final word; prolonged search, examination of the visceral blood, and especially a cyto-index, would have converted many of these negatives into positives.

Results	Benign parasites.. ..	66·7 per cent.
	Malignant parasites ..	28·6 ,,
	Unclassified parasites ..	4·7 ,,

It is important to note that the per mille rates given are those which would have obtained had the admission-rate for the period of quinine prophylaxis been continued for the year. They have been adopted as the only means of rendering comparable the varying periods of prophylaxis which have been adopted in different stations.

Quinine was in all instances given in solution, thus obviating any idea that unassimilable tabloids were used. It was given at compulsory parades, carried out under regimental orders, and absentees were reported by the assistant surgeon or medical officer who administered the dose. In some instances, the medical officer (as at Delhi) secured that it was not only taken, but swallowed, by making the recipient answer to his name after taking the dose. There is thus no reason for doubting that the prophylaxis was maintained.

The dosage and intervals adopted were:—

10 gr. on two consecutive days per week = Delhi, Muttra, Agra and Roorkee.
10 ,, at three-day intervals .. .. = Bareilly, Delhi and Meerut.
10 ,, at two-day intervals .. .. = Dehra Dun.
15 ,, on two consecutive days per week = Dehra Dun.

from which it is seen that a thorough and varied trial was made.

RESULTS OF PROPHYLACTIC QUININE ON MALARIA ADMISSION RATES OF SOME STATIONS IN THE 7TH DIVISION, IN 1909.

Station	Strength	Number of days' quinine prophylaxis	Admissions (actuals)	Admissions per mille for a year at the same rate
Bareilly	1,024	60	148	878
Dehra Dun	625	120	140	672
Delhi	497	270	370	1,016
Muttra	488	150	14	73
Roorkee	308	90	69	908
Meerut	1,192	60	186	960
Agra Fort	350	270	281	1,088

These figures were so opposed to one's preconceived ideas of the value of quinine prophylaxis, and present so overwhelming a case against its use, that hesitation was felt in accepting them. It was feared that our precautions must have left some loophole for error, and hence it was decided to collect similar data for another year as a control.

It was also noted that Muttra was the only station in which the results were acceptable as conforming in any degree to our

expectations. Muttra reported, moreover, that the regiment there had spent considerable sums on the surface drainage of the station, while prophylactic quinine had not only been administered to the men, but to all families and followers, even including private servants in officers' bungalows. That note was communicated to the other stations, and Roorkee replied that they had experienced far better results since they had adopted similar thorough measures. It was thus felt desirable that the controls for 1910 should be carried out on similar lines, including the administration of quinine to all followers and their children, both in the lines and as far as possible in the bazaars also.

It is important to add this most relevant remark, by the Senior Medical Officer at Muttra, *re* the energy spent on surface drainage and anti-mosquito measures, " . . . the paucity of mosquitoes was noticeable."

Another important point which is brought out is that the benign malarial parasites formed 66·7 per cent of the total malarial parasites found in the blood of men taking prophylactic quinine regularly. This is, perhaps, the most significant fact that we have to face.

RESULTS OF PROPHYLACTIC QUININE ON MALARIA ADMISSION RATES OF SOME STATIONS IN THE 7TH DIVISION IN 1910.

Station	Days of quinine prophylaxis	Admissions per mille per annum—if rate of period continued	Diagnoses proved	Benign parasites
Delhi ..	230 ..	796 ..	56 per cent ..	78 per cent
Roorkee ..	141 ..	315 ..	92 ,, ..	81 ,,
Muttra ..	140 ..	635 ..	56 ,, ..	98 ,,
Agra ..	148 ..	639 ..	39 ,, ..	91 ,,

Note 1.—One Native Infantry Regiment at Delhi gave admissions for malaria at the rate of 1,071 per mille for the prophylactic period of 214 days.

Note 2.—Quinine was administered in solution in all cases as follows: Delhi and Roorkee, 10 gr. on two successive days per week under the supervision of the medical officer at Delhi, also an extra 5 gr. was given on the intervening days of the worst period (*vide infra*). Under the supervision of an assistant surgeon all followers and children were given equivalent doses, and at Roorkee those in the bazaars as well as those in the lines.

Muttra.—Fifteen grains once a week to all strength and followers. The Senior Medical Officer remarks, " I sometimes saw it made up, and always saw it administered, while I invariably looked for iridescence in the mixture before it was administered."

Agra.—Ten grains on Thursdays and Sundays, which was changed during the worst period for a daily dose of 5 gr. Followers were similarly treated from July 14 to October 9, while children got from 3 to 5 gr., both in the lines and bazaars.

There appears no doubt that every means was taken in these stations to secure the success of this method of prevention.

Note 3.—The increase in the percentage of benign parasites in the blood of the persons so dosed with quinine is noted specially.

Note 4.—The percentage of cases returned as "initial attacks" is very high, but, pending adoption of the malaria case-sheets recently ordered, this value is only of relative importance. The percentages worked out thus: Delhi, 91 per cent; Roorkee, 82 per cent; Muttra, 22 per cent; Agra, 56 per cent.

Generally it may be remarked that these extended observations fully confirm the approximate accuracy of those of the previous year. The hope that more universal dosage of followers with quinine would produce results elsewhere comparable with those obtained at Muttra, has proved vain—in fact, Muttra, in spite of all its zeal, shows an admission-rate which has risen from 73 to 635 per mille since the previous year.

Measures have been organized by the Principal Medical Officer of this division to ensure complete returns of similar data, which will be available at the end of the present year. Meanwhile he is of opinion that the first figures and their controls are so obviously confirmed and so universally important that they should not be withheld longer.

In considering their bearing and significance, it will be better to concentrate on one station—Delhi—which will give ample material for careful consideration and anxious thought.

Delhi.—(1) Has quinine been taken?

(*a*) With regard to the troops—yes. It has always been administered in the presence of the medical officer, who has made each man answer his name after taking the dose into his mouth. Absentees from the quinine parades have invariably been reported, and with rare exceptions brought up for their dose next day. Quinine has been given in various ways: on two consecutive days per week, every third day, every day and, finally, small daily doses combined with larger doses twice a week.

(*b*) With regard to followers, there has been difficulty. It is not an easy matter to get at the purdah families, but a determined attempt has been made, and it has proved generally successful by mingled persuasion, tact, and threats of eviction. The punkah

coolies have proved intractable however—they refused to take quinine; the contractor could not replace them with men who would take it; 50 per cent of them had enlarged spleens. Capital probably will not be made out of this fact when we come to consider the presence of malarial parasites in the blood of those who undoubtedly were getting quinine.

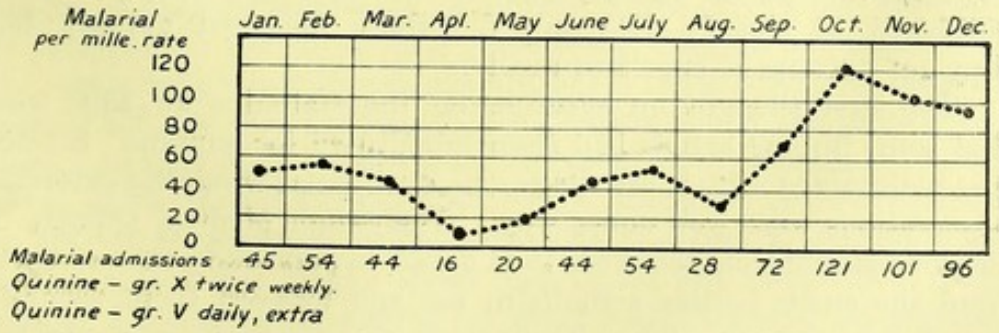
(2) Value of the statistics.

The opinion of the Senior Medical Officer—quoted on p. 464—shows that he brought to the study of this matter a belief in prophylactic quinine, and his confession of disappointment adds point to the statement that everything was done to make it a success, as well as to the guarded admission that it has done little good.

Overwhelming as the admission-rates in the above tables appear, they do not represent anything like the full extent of malarial prevalence. In 1909, the malaria admission-rate for the British garrison of Delhi amounted to 1,144 per mille, but there were also 181 admissions for "pyrexia of uncertain origin," which shows that no clinically uncertain cases were returned as malaria, while over half the cases were proved by microscopic examination to have parasites in their blood. To the above enormous rate we have to add the malarial attacks treated without admission to hospital. For the whole of India the Sanitary Commissioner in 1909 gave the ratios as respectively 202 : 96, while in Delhi itself in September of that year an independent observer (Lieutenant-Colonel P. Hehir, I.M.S.) reported that "It is considered that detentions to actual admissions are as $3\frac{1}{2}$: 1." The former estimate makes the malarial attacks 1,700, and the latter makes them 4,000 per mille per annum.

(3) The relation between the relative amounts of malaria and prophylactic quinine is best shown by the following chart for 1910:—

CHART IV.



(4) The malarial conditions characterizing Delhi Fort.

It is fortunate that these have been investigated by two quite

unbiased observers, whose standing is sufficient authority for their findings. One was Lieutenant-Colonel P. Hehir, I.M.S., and the other Lieutenant-Colonel J. R. Adie, I.M.S., both specially selected for malarial investigation work. The data and quotations in this section are from the reports of these officers.

September, 1909.—Anopheles larvæ were found in each of twenty-nine bela pools examined; the imagines were carried thence into the fort by the prevailing wind.

Of the *Myzomyia culicifacies* captured in the fort, 35 per cent harboured malarial parasites.

Of the followers' children in the casement quarters, 58 per cent of those examined had malarial parasites in their blood.

November, 1910.—Of the followers' children in the fort, 43 per cent of those examined had malarial parasites in their blood.

Of 150 men of the garrison doing duty and not on the sick list, "More than a fourth are carrying about malarial parasites in spite of the large amount of prophylactic quinine that they are taking. It is a most interesting and surprising investigation." "It seems to me that, pending improvements of drainage, you must go in for more and more *efficient* mechanical protection. I doubt if you can give much more prophylactic quinine and expect the men to do their ordinary round of duty." In other words this means that quinine prophylaxis has hopelessly broken down and that, if pressed further, it will break down the garrison also—truly an inspiriting expert opinion for the advocates of this method to ponder upon! So far as this series of observations is concerned, quinine prophylaxis not only fails to justify its ambitious claims of rendering persons "absolutely malaria-proof," but is very hard put to it for ground which will even justify its existence.

Quality of the quinine used during the periods under review.—This appears to be the only avenue of retreat left open; it must ruthlessly be closed. Not only did these men get quinine, but they got quinine of excellent quality.

The first thought on scrutinizing the statistics for 1908 was, that some impure article had been substituted for quinine. Silence was maintained as to these suspicions until later, when the extended observations were well under way. The Senior Medical Officers of the four stations chosen were then asked to personally take samples from the stock bottles actually in use and forward them, securely sealed, for analysis. They kindly took these samples and adopted full precautions as requested.

The full results had better be appended as showing the general

accuracy and completeness of the analysis. Owing to limitations of the laboratory, only three samples and the standard could be tested at the same time. The samples thus analysed were from Delhi, Muttra and Agra.

The quinine sulphate was separately estimated by acidimetric and gravimetric methods. The greatest difference in the results by the two methods was 0·6 per cent, and the gravimetric result was higher in each case. The mean of these two estimations is given in the appended table of results.

Results of Analyses of Three Quinine Samples.

	Standard	Sample I.	Sample II.	Sample III.
Quinine sulphate	97·60	98·40	97·40	97·65
Cinchonidine	·12	·14	·12	·18
Quinoidine and Cinchonine ..	trace	·05	trace	trace
Ash	·15	·21	·26	·22
Water	2·60	2·10	2·50	2·45
	100·47	100·90	100·28	100·50

It is seen that the analytical error in no instance amounted to as much as 1 per cent ; the variation in the result is probably largely due to difficulty in obtaining absolute accuracy by the gravimetric method.

Lastly, what evidence is there of the effect of the administration of prophylactic quinine upon the amenability of subsequent malaria to the curative action of that drug ?

Statistics have been kept which show the relapses occurring among a total of 1,053 malarial patients who attended hospital at Meerut for three months subsequent to their discharge. During that period they received 15 gr. of quinine on two days per week. The drug was administered in solution and in the presence of an assistant surgeon, who kept a roster of these men and strictly enforced their regular attendance. It is unfortunate that the information cannot be expressed in precise terms of the per mille rate of relapses per annum among these men. We do not know what the mean daily strength was exactly, but it must have been approximately 170, for the eighteen months during which the results were kept. In that period there were 734 recurrences among these men —i.e., at the rate of 489 per year. It is, of course, obvious that this is but an approximate calculation—but it shows a per mille per annum attack rate among these men of no less than 2,876, which affords a considerable margin for error without losing its startling character.

Within comparatively recent years it has been taught that the

action of quinine upon malarial parasites was so certain that quinine could be relied upon for clinical diagnosis. Was that teaching correct? Has quinine lost that specific curative action? If so, is that loss due to changes in the resisting powers of the parasite? If so, has the parasite become quinine-fast? If so, is that in any degree the result of constant habituation to small doses of quinine given prophylactically? These are questions of the utmost practical importance, and, moreover, of an urgent importance. They are questions to which everyone who has the health of the Army in India at heart wants an authoritative answer. They are questions compared with which those of the time, energy, and money spent on quinine prophylaxis sink into relative insignificance.

In submitting this criticism it is not suggested that quinine prophylaxis, if properly safeguarded, has no value under any circumstances; but it is essential that a protest be made against the one-sided view, that in the wholesale administration of prophylactic quinine we have a simple and royal road to malaria prevention, warranting the withdrawal or omission of all other measures. In the light of the foregoing data, no more dangerous doctrine than this could be advocated, yet its advocacy by their medical advisers has been seized upon by the civil administration as an argument for abandoning more radical measures. It is hoped that this critical analysis of official statistics may draw attention to a most important matter, and enable some of the facts concerning it to be seen in their proper perspective.

AN OUTBREAK OF PARATYPHOID B FEVER IN MALTA.

By Major M. H. BABINGTON.
Royal Army Medical Corps.

Towards the end of August, 1910, there was a sudden outbreak of fever in the 1st Battalion Suffolk Regiment. The outbreak was preceded by a few isolated cases earlier in the year. The first case was admitted to hospital on May 29, 1910, from St. Andrew's Barracks. He was discharged to his barracks on July 25, 1910, his excreta having been examined and found free from infection. Sixteen days after this patient's discharge, Private L., the second case, felt ill. The intervals in days between the return of the first case to barracks, and the onset of the disease in the succeeding cases, i.e., the second to the fourteenth in the series, were as follows: 16, 25, 29, 30, 31, 32, 32, 31, 33, 33, 34, 36, 51. All the cases but one came from the Suffolk Regiment occupying St. Andrew's Barracks, the exception was Case No. 11, who belonged to the King's Royal Rifle Corps living in the adjoining St. George's Barracks.

The table below shows the cases in the order in which they were admitted to hospital. The temperature charts are those of some of the cases in which the bacillus was found in the blood. The number on the temperature chart refers to the number of the case in the table.

Clinical Notes on the Cases.

Case No 1.—Private D. resembled an ordinary mild enteric. Blood culture 5 c.c. in ox bile remained permanently sterile. There was no diarrhœa. Epistaxis was noticed on the fifth and eighteenth days. Convalescence was protracted by a weak and rapid heart.

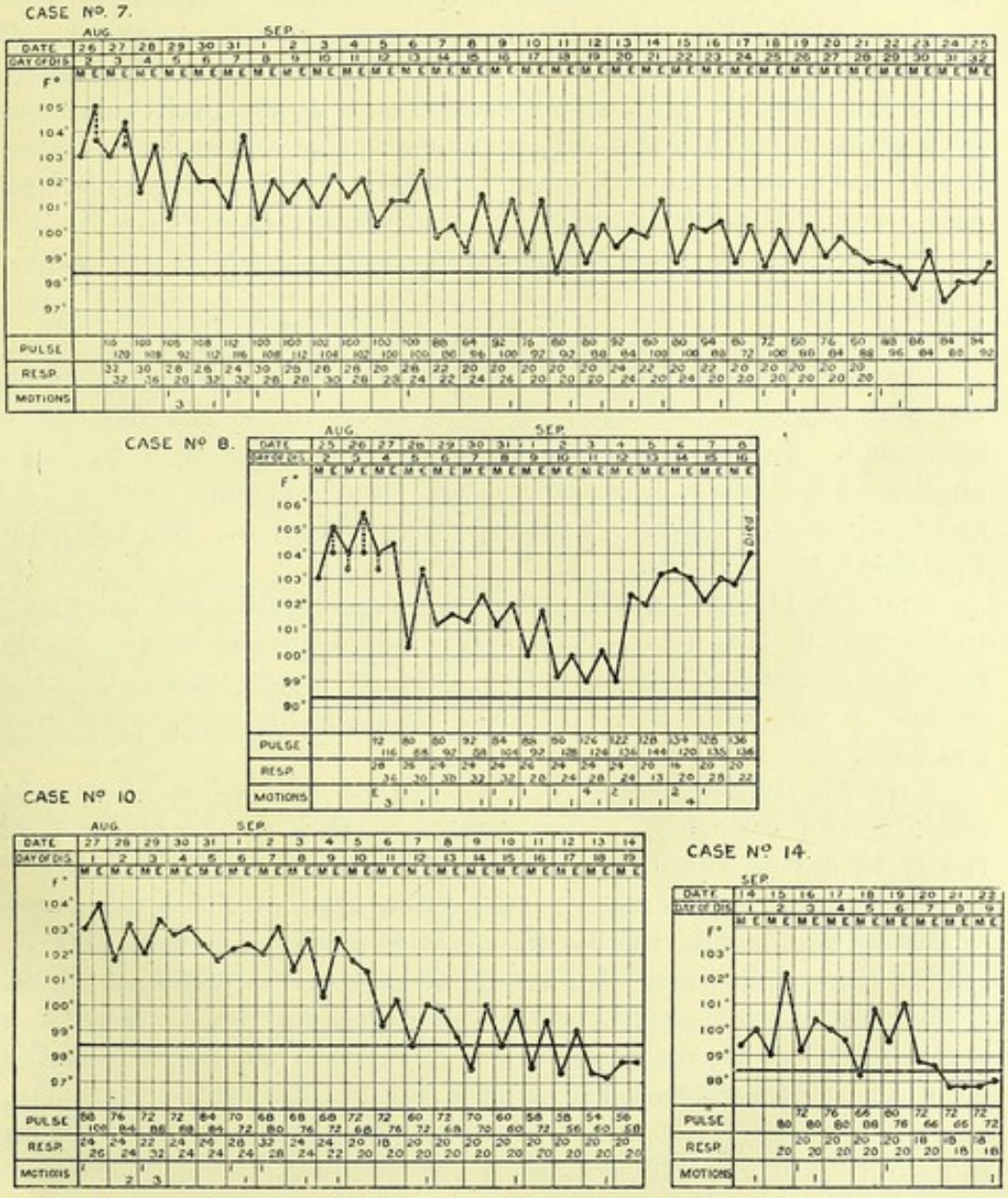
Case No. 2.—Private L. felt remarkably well during the attack, the only symptom being headache during the first few days. For four days the pyrexia was continuous. For the remaining eighteen days temperature was intermittent, never rising much above 101° F.

Case No 3.—Private F., practically the only symptoms were slight headache and malaise. The temperature seldom exceeded 100° F., and was intermittent throughout.

No.	Rank	Name	Corps	Quarters	Onset	Admitted	Discharged	Duration of pyrexia in days	Result of blood culture	Serum reactions	
										Para. B	B. typhosus
1	Pte.	D. ..	Suffolk Regt. ..	St. Andrew's	4.5.10	6.5.10	25.7.10	23	Sterile	+ 1-80	+ 1-20
2	,,	L. ..	,, ..	,, ..	10.8.10	12.8.10	29.9.10	22	None taken ..	+ 1-2560	− 1-20
3	,,	F. ..	,, ..	,, ..	19.8.10	20.8.10	27.9.10	16	,, ..	+ 1-1280	− 1-20
4	,,	G. ..	—	—	23.8.10	24.8.10	28.9.10	12	,, ..	+ 1-320	− 1-20
5	Cpl.	B. ..	,, ..	St. Andrew's : 1 Room, D Block	24.8.10	27.8.10	7.10.10	20	Para. B grown	+ 1-640	± 1-20
6	Pte.	K. ..	,, ..	St. Andrew's	25.8.10	27.8.10	4.10.10	9	Sterile	+ 1-320	− 1-20
7	,,	N. ..	,, ..	St. Andrew's : 2 Room, C Block	26.8.10	27.8.10	16.11.10	30	Para. B grown	+ 1-160	± 1-20
8	,,	T. ..	,, ..	St. Andrew's : 3 Room, C Block	26.8.10	27.8.10	9.9.10 (Died)	16	,, ,,	+ 1-320	± 1-20
9	Sgt.	S. ..	,, ..	St. Andrew's : Bunk, 3 Room, C Block	25.8.10	29.8.10	28.9.10	16	,, ,,	± 1-40	− 1-20
10	Pte.	H. ..	,, ..	St.Andrew's : 2 Room, A Block	27.8.10	27.8.10	12.10.10	18	,, ,,	+ 1-1280	± 1-20
11	,,	T. ..	K.R.R ..	St. George's	27.8.10	27.8.10	28.9.10	5	None taken ..	+ 1-160	− 1-20
12	Cpl.	A. ..	Suffolk Regt. ..	St. Andrew's : I Block	28.8.10	29.8.10	4.10.10	12	Para. B grown	+ 1-640	± 1-20
13	,,	G. ..	,, ..	,, ...	30.8.10	30.8.10	20.9.10	4	None taken ..	+ 1-320	− 1-20
14	,,	C. ..	,, ..	St. Andrew's : 3 Room, H Block	14.9.10	14.9.10	12.10.10	6	Para. B grown	—	—

Case No. 4.—Private P. was similar to No. 3.

Case No. 5.—Corporal B. admitted on the fourth day of his disease with a herpetic eruption on the right side of his mouth. He suffered from persistent headache for some days, otherwise no special symptoms.



Case No. 6.—Private K. Temperature 102·8° F. on admission. Had been ill for three days before coming to hospital. Had irregular intermittent fever for nine days. The only symptoms were headache, retention of urine, and pain over liver.

Case No. 7.—Private N. after admission became rapidly delirious and semi-conscious. Abdomen distended, and face cyanosed. Began to improve on sixth day. A very profuse rash appeared on eighth day. Thrombosis of long saphenous vein supervened on the twenty-second day. The rash was composed of spots resembling enteric spots. See chart, Case No. 7.

Case No. 8.—Private T. became delirious on the seventh day of his illness. Hæmorrhage from the bowel occurred on the eighth, tenth and twelfth days. The hæmorrhage on the twelfth day was very profuse, leaving the patient almost pulseless, and necessitating the use of subcutaneous saline infusions. This was the only fatal case. See post-mortem report and temperature chart, Case No. 8.

Case No. 9.—Serjeant S., a mild case. Rash, which was scanty, appeared on the sixth day and resembled a typical enteric rash, After the first few days patient had no symptoms.

Case No. 10.—Private H., a moderately severe case admitted on the first day of the disease. Felt perfectly fit on the day before admission. Suffered from severe pain in the muscles of the back on the fifth, sixth and seventh days. Had epistaxis on the first and tenth days. Numerous rose spots on abdomen. See temperature chart, Case No. 10.

Case No. 11.—Private T.; this was the only case not belonging to the Suffolk Regiment. The diagnosis was based on the serum reaction, and on the recovery of the bacillus paratyphosus B in the fæces. A very mild case. Pyrexia, remittent in type, lasted only five days.

Case No. 12.—Corporal A., diarrhœa on admission, afterwards retching and vomiting. Very numerous rose spots appeared on the third day of the disease. A mild case.

Case No. 13.—Private G., the mildest case in the series. Admitted on the first day of his disease with a temperature of 102° F. On the three following days temperature rose to just over 99° F. Only symptoms were thirst and slight abdominal pains. Serum on the third day of the disease in a 1 in 10 dilution was negative to the paratyphoid strain isolated from Case No. 10; but on the ninth day, i.e., five days after his temperature had become normal, the serum in a dilution of 1 in 320 completely agglutinated the same strain.

Case No. 14.—Private C., practically the only symptoms were pyrexia and epistaxis. See temperature chart, Case No. 14.

Diagnosis.

In seven cases the diagnosis was made on the result of the blood culture. In the remaining cases the diagnosis was based on

the serum reactions. (See Table.) The strain of paratyphoid B bacillus used for this purpose was that isolated from Case No. 10. This strain proved to be quite reliable for this purpose. Normal serums in a dilution of 1 in 10 failed to cause any agglutination with emulsions of this bacillus, and serum in a 1 in 20 dilution from undoubted cases of enteric fever caused only incomplete agglutination. Since this epidemic I have put up numerous samples of serum with emulsions of this bacillus but have never found one to agglutinate it. For statistical purposes only the seven cases from whose blood the bacillus was isolated were returned as paratyphoid B fever.

Post-mortem Notes.

Extract from post-mortem report of Case No. 8. "The mucous membrane of the duodenum and jejunum appears healthy, but in the ileum the intestinal wall is thin and atrophied. Peyer's patches are very distinct but show no signs of inflammation or of ulceration. The large intestine is intensely inflamed throughout its whole length. The mucosa is deep red in colour and studded thickly from the ileocæcal valve to the anus with innumerable ulcers varying in size from a pinhead to a sixpence. The ulcers are largest in the cæcum and the necrosis is here very deep, extending to the peritoneum. The mucosa of the cæcum is almost completely destroyed by ulceration, what remains is of a greenish colour and appears to be gangrenous."

Details as to Previous Inoculation against Enteric Fever.

Case No. 5.—Received the following doses of antityphoid vaccine.

November 7, 1907, 0·45 c.c.; November 22, 1907, 1·0 c.c.; March 29, 1910, 0·5 c.c.; April 16, 1910, 1·0 c.c.

Case No. 7.—Was inoculated with only one dose at Woolwich, in 1907.

Case No. 8.—A fatal case. Had not been inoculated against enteric.

Case No. 9.—Had not been inoculated against enteric.

Case No. 10.—Inoculated in February, 1910, receiving two injections.

Case No. 12.—Had not been inoculated.

Case No. 14.—Received one injection at Woolwich, 1907, also one injection at Malta, 1909.

Cause of the Outbreak.

As the cases came from a considerable distance I was unable to investigate the epidemic on the spot. The regiment had a good sanitary reputation, in fact, in attention to sanitary detail it was considered second to none on the island. It will be noted that the first case occurred in May, and that there were no other cases until after the first had returned to barracks. This first case was a married man living in quarters; on his return to barracks he was in no way concerned in the preparation of food; so that it is difficult to imagine in what manner he could be responsible for the outbreak. A spot map showed that the cases came from no particular room or block of buildings. The fruit contractor to the regiment was discovered to have his fruit stored outside of barracks in a very filthy room, in which he also kept pigs. This was considered to be the source of infection from which the disease originated. Many of the patients, however, did not eat this fruit. Several of them stated that they had never eaten fruit in Malta. The fruit therefore could not have been an important factor in disseminating the disease. The barracks have a water-carriage system of sewage disposal. The water supply is that known as the Wigancourt and was being used without bad effects by several units, moreover for six months before the outbreak all drinking-water had been boiled.

Bacteriology.

Blood cultures were made from nine of the cases. From seven of these the paratyphoid B bacillus was isolated—two of the cultures remained permanently sterile. The method employed in the majority of the cases was as follows: 10 c.c. of blood was withdrawn by means of an antitoxin syringe from a vein at the bend of the elbow, and introduced into four test-tubes, each containing 12 c.c. sterile ox bile. One cubic centimetre of the blood was put in the first tube, 2 c.c. in the second, 3 c.c. in the third, and 4 c.c. in the fourth. After about sixteen hours at 37° C., agar slopes were inoculated. Hanging-drops made about six hours later from the agar slopes showed numerous bacilli when the blood culture was successful. In two cases the method of adding 2½ c.c. of blood to 250 c.c. of sterile water was tried, and in both cases successful results were obtained, but more time was required than with samples of the same blood in ox bile.

The biochemical reactions of each culture were then examined, and a diagnosis made. By this means it was possible to say thirty-four hours after making a blood culture that the cases were not enteric,

and at the end of three days, when the litmus milk had become definitely alkaline, the cases were diagnosed paratyphoid B fever.

In October, 1910, Major W. S. Harrison wished to examine the culture isolated from the blood of the fatal case. I was fortunately able to accede to his request for a sub-culture. This he examined, and found it to be paratyphoid B. All the cultures but one which was being frequently sub-cultured for use in the laboratory gradually died out. This one was obtained from Case No. 10 in the series, and has been sub-cultured on agar at intervals of a month since it was first isolated.

After reading the articles which have appeared recently in the JOURNAL OF THE ROYAL ARMY MEDICAL CORPS, I thought it advisable to have an unbiassed opinion on this bacillus. I therefore sent a culture of this organism, isolated from Private H., Case No. 10, to the Royal Army Medical College, giving no information as to its source, and requesting that I might be informed whether it was paratyphoid A or B. This investigation was carried out by Major S. L. Cummins, whose report I append.

"REPORT BY MAJOR S. L. CUMMINS ON CULTURE SENT BY MAJOR BABINGTON FROM MALTA—SOURCE NOT STATED.

"*Morphology.*—Short motile bacillus; negative to 'Gram.'

"*Cultural Characters.*—Forms acid and gas in glucose, mannite, maltose, and dulcite; no change in lactose, cane sugar, salicin, and inulin. Litmus milk, in twenty-four hours, slightly acid; in three days, alkaline. Indol *nil*, or slight trace in nine days.

"*Agglutination.*—Unaffected by paratyphoid A serum (Lister); agglutination complete to $\frac{1}{200}$ (one hour), with antityphoid serum (Burroughs Wellcome and Co.).

"*Absorption.*—It removes from a typhoid serum the group agglutinins affecting paratyphoid B; and paratyphoid B removes from a typhoid serum the agglutinins affecting the 'No. 10' strain.

"*Further Tests.*—(1) A rabbit immunized against the 'No. 10' strain developed agglutinins for both 'No. 10' and paratyphoid B.

"(2) A rabbit immunized against paratyphoid B, agglutinated both paratyphoid B and the 'No. 10' bacillus.

"(3) The 'No. 10' strain absorbed all agglutinins both for Paratyphoid B and the 'No. 10' strain.

"(4) Paratyphoid B absorbed all agglutinins both for 'No. 10' bacillus and paratyphoid B.

"Tests '3' and '4' were carried out with both sera.

"There is no doubt that 'No. 10' strain is a true paratyphoid B."

John Bale, Sons & Danielsson, Ltd., 83-91, Great Titchfield Street, London, W.

FURTHER INVESTIGATIONS ON THE USE OF SALVARSAN IN SYPHILIS.

By Major T. W. GIBBARD, Major L. W. HARRISON and Lieutenant A. S. CANE.

Royal Army Medical Corps.

Sufficient has been written by ourselves and others to show that salvarsan has a more rapid effect than mercury on the symptoms of syphilis and that it almost always succeeds where mercury has failed to arrest the progress of syphilitic lesions. We have also shown by its effect on the Wassermann reaction and in causing *Spirochætæ pallidæ* to disappear from such lesions as chancres and condylomata that it is not merely a symptomatic remedy, but a specific one, and that in this respect its action is much more intense than that of mercury. Though we have recognized, with everyone, the very great advantage which would accrue if salvarsan could completely replace mercury, and our investigations have been directed towards ascertaining if this is possible, our results have not so far justified us in saying that treatment by salvarsan alone is sufficient to insure the recovery of every case of syphilis. As we have mentioned in previous papers, we have tried the effect of one injection, intramuscular or intravenous, two intravenous and four intravenous injections respectively on successive groups of cases, our aim being to find the simplest and most effective method of using salvarsan. Some clinical relapses have occurred, however, in each of the four groups and in more cases the Wassermann reaction has either remained persistently positive or has again become so after being converted to negative, so that we cannot say we have yet succeeded in discovering a method of using salvarsan which will invariably insure the destruction of every *S. pallida* in the patient's body.

As it seemed to us probable that relapses after salvarsan treatment were not due to the generation of salvarsan-resistant strains of *S. pallida*, seeing that further treatment of relapse cases with this remedy was just as effective as in the first instance, and and as we inclined to the belief that the first injection of salvarsan did not reach every parasite on account of some being buried in thrombi, &c., we determined to try the effect of an initial full dose of salvarsan followed by nine injections of mercurial cream and, lastly, another full dose of salvarsan. Our object was to destroy every *S. pallida* which could be reached by salvarsan and to

commence a process of repair in sclerosed areas ; to keep up a constant attack with mercury on the previously buried parasites as they became exposed by the vascularization of thrombi, so as to prevent them from causing any more scleroses, and, finally, to destroy them with salvarsan. We have treated eighty-five cases in this manner, but though no relapses have occurred in this group, sufficient time has not yet elapsed to enable us to compare it fairly with others.

Other workers, notably Gennerich and Neisser, recommend the combined use of salvarsan and mercury, and it seems very probable that a method of treatment on these lines will be that adopted in future.

We propose in this paper to indicate, as far as we are able at present, the effect which the more general use of salvarsan is likely to have on the treatment of syphilis in the Army, to mention some points of importance in the technique of administration and to outline a preliminary scheme for the management of cases treated with salvarsan.

Regarding the effect which salvarsan is likely to have on the treatment of syphilis, it is necessary to compare the behaviour of this disease under purely mercurial treatment with that when salvarsan is used, either alone or in combination with mercury, as above mentioned. For this purpose we have examined our notes on such of our cases as had received no treatment whatever up to the time of the first injection of salvarsan and were subsequently kept under observation for four, six and nine months respectively, and have recorded in each case (*a*) whether any recurrence of symptoms occurred after the lesion for which the patient was admitted had healed, and after what interval from the date of the first injection ; (*b*) the average length of stay in hospital on first admission, and (*c*) the average length of stay in hospital on re-admission for relapse. We had eighty-eight cases under observation for four months, seventy-five of these were under observation for two months longer, and forty-five of these were observed for nine months in all. In every case the diagnosis was absolutely assured by combined clinical and laboratory examination.

For comparison with the behaviour of these salvarsan cases, we chose at random eighty-eight recent syphilis case-sheets of patients, treated with mercury only, and extracted from them similar particulars relating to a period of four months ; from the first seventy-five for a period of six months and from the first forty-five for a period of nine months.

The result of the examination is shown in Table I, from which it will be seen that the salvarsan cases not only suffered considerably less from recurrence of clinical signs, and consequently spent a much shorter total time in hospital, but enjoyed a much greater interval of freedom between the first and any further outward manifestations of the disease.

TABLE I.—To Illustrate the Effect of Salvarsan and Mercury respectively in Preventing or Postponing the Recurrence of Active Signs in Syphilis.

Cases under observation for the following periods from the commencement of treatment	Total cases under each kind of treatment	Relapsed within four months		Relapsed between four and six months		Relapsed between six and nine months		Total number of relapses within each of the periods stated		Average interval in weeks between commencement of treatment and first relapse		Average duration of stay in hospital on first admission, in days		Average duration of stay in hospital on re-admission for first relapse, in days	
		"606"	Hg	"606"	Hg	"606"	Hg	"606"	Hg	"606"	Hg	"606"	Hg	"606"	Hg
4 months ..	88	4	68	..	..	..	..	4	68						
6 ,, .	75*	4*	57*	3	6	..	..	7	63	20·5†	9·25‡	21·5	27·9	15·6	21·7
9 ,, ..	45*	4*	35*	2*	5*	4	1	10	41						

* Each of these numbers is included in those above it in the same column.
† Average calculated from all available cases, viz., 11.
‡ ,, ,, ,, the 75 cases which relapsed.

It is not sufficient, however, that we should merely state the bare numbers under each of the headings we have mentioned; to obtain a correct estimate of the different effects of the two methods of treatment it is also necessary to give particulars of the treatment administered, especially in the case of those patients who were treated with mercury. The result of our scrutiny showed the following:—

I. (*A*) Of the 88 cases treated with mercury and observed for four months:—

(1) Sixty-nine were treated with regular injections of mercurial cream (Hg. gr.i in each), salicylate of mercury (gr.1·5 in each), or partly calomel cream (Hg_2Cl_2 gr.$\frac{3}{4}$ in each) and partly mercurial cream, and 52 of these relapsed at the following periods:—

1	after the 3rd injection			3	after the 8th injection		
3	,,	,, 4th	,,	12	,,	,, 9th	,,
10	,,	,, 5th	,,	3	,,	,, 11th	,,
13	,,	,, 6th	,,	1	,,	,, 12th	,,
6	,,	,, 7th	,,				

Of those cases which relapsed subsequently to the sixth injection, in 8 the first course consisted of 6 injections only; of those which relapsed subsequently to the seventh injection, in 1 the first course consisted of 7 injections only; of those which relapsed subsequently to the eighth injection, in 2 the first course consisted of 8 injections; with these exceptions all who relapsed subsequently to the ninth injection received 9 weekly injections in the first course.

The 17 cases under this heading which did not relapse within four months were treated as follows during that period: 2 had a course of 6 weekly injections, a rest of four weeks and then 4 more injections; 10 had a course of 9 weekly injections and no further treatment till the end of the four months; 1 had a course of 10 weekly injections and no further treatment till the end of the four months; 1 had a course of 11 weekly injections and no further treatment till the end of four months; 2 had a course of 9 weekly injections, a rest of four weeks and 2 fortnightly injections; 1 had a course of 9 weekly injections, a rest of six weeks, and 1 further injection.

(2) Five cases were treated with inunctions only, and all relapsed within the four months. Particulars of their treatment up to the date of relapse were as follows: 1 had received 12 out of a course of 42 daily inunctions at the Royal Herbert Hospital, Woolwich; 1 had received 15 inunctions at Windsor; 1 had received 26 daily inunctions at Woolwich, was transferred to another station, and treatment omitted till he relapsed two months later; 1 received 40 daily inunctions at the Royal Herbert Hospital, was recommended "two months' interval," and relapsed eight weeks later; 1 had 42 daily inunctions at the Royal Herbert Hospital and relapsed five weeks later.

(3) The remaining 14 of the 88 cases were treated partly by inunctions and partly by injections, and 11 of these relapsed after receiving an average of 13 daily inunctions and 4 weekly injections.

The 3 cases which did not relapse within four months received treatment as follows during this period: 2 had 40 and 42 inunctions respectively at the Royal Herbert Hospital, a rest of four to six weeks, and then 4 injections; 1 had 12 inunctions in South Africa and then 6 weekly injections.

(*B*) Of the 75 cases which were treated with mercury and observed for six months, 63 relapsed within this period; the treatment of 57 which did so within four months has already been

detailed. The treatment of the remaining 6 cases up to the date of relapse was as follows: 1 had a course of 9 weekly injections, and relapsed at the end of the two months' rest which followed; 1 had 11 weekly injections and relapsed at the end of two months' rest; 1 had 9 weekly injections, two months' rest and 1 injection of the second course; 1 had 9 weekly injections, two months' rest and 2 injections of the second course; 1 had 9 weekly injections, two months' rest and 3 injections of the second course; 1 had 42 daily inunctions at the Royal Herbert Hospital, six weeks' rest and 4 injections of the second course.

Of the 12 cases in this group which did not relapse within six months, 10 received 9 weekly injections, six to eight weeks' rest and a second course consisting of 3 to 6 fortnightly injections; 1 had 6 daily inunctions at the Royal Herbert Hospital and 6 weekly injections in the first course, two months' rest and 4 fortnightly injections of the second course; 1 had 12 daily inunctions, followed by 6 weekly injections in the first course, two months' rest and then 4 fortnightly injections in the second course.

(*C*) Of the 45 cases treated with mercury and observed for nine months, 40 relapsed within six months, and their treatment has already been detailed. Of the remaining 5 cases, 1 relapsed between six and nine months and was treated as follows up to the date of the relapse: 10 weekly injections, six weeks' rest, 6 fortnightly injections and three weeks' rest; the 4 cases in this group which did not relapse at any time during the nine months were treated as follows: 1 had 9 weekly injections, eleven weeks' interval, and then 6 fortnightly injections; 1 had 4 inunctions followed by 6 weekly injections in the first course, two months' rest, 4 fortnightly injections, two months' rest and 1 injection of the third course; 1 had 9 injections in the first course, two months' rest, 6 fortnightly injections in the second course, and then a rest; 1 had 9 weekly injections, five weeks' rest, 6 injections in the second course, and then a rest. It will be recognized, therefore, that in the very great majority of the mercurial cases mercury was given a fair trial.

II. The salvarsan cases were treated as follows: 16 had an initial intra-muscular injection of 0·6 grm; all were under observation for the full nine months, and 2 relapsed within four months; 1 between four and six months and 1 between six and nine months: 6 had a single intravenous injection of 0·5 to 0·6 grm.; all were under observation for nine months, and 2 relapsed within four months; 1 between four and six months and none between six and nine months: 20 had two intravenous injections of 0·4 to

0·6 grm. at intervals of two weeks; all were under observation for nine months, and 2 relapsed between six and nine months: 34 had an initial intravenous injection of 0·5 to 0·6 grm. followed by 3 intravenous of 0·2 to 0·3 grm. at fortnightly intervals. All of these were under observation for at least six months; 9 for at least nine months; 6 for at least eight months; and 12 for at least seven months: 1 relapsed between four and six months and 1 between six and nine months: 12 had an initial intravenous injection of 0·6 grm., 9 weekly injections of mercurial cream (Hg. gr.i in each) and, finally, an intravenous injection of 0·6 grm. salvarsan: 6 of these were under observation for at least six months and 6 for at least five months; none relapsed.

Of the cases which did not relapse, eight were subsequently given an intravenous injection of 0·6 grm. salvarsan on account of the Wassermann reaction being positive. Excepting local treatment to primary sores, this was all the treatment which the respective cases received.

TABLE II.—To Illustrate the Effect of Salvarsan and Mercurial Treatment Respectively in Preventing or Postponing the Occurrence of Secondary Symptoms when Treatment is Commenced in the Primary Stage.

Method of treatment	Total cases under each treatment	Number which subsequently developed secondaries	Per cent. which developed secondaries	Remarks
Salvarsan alone or combined with mercury*	38	2	5·2	*Nine were treated with a combined course of salvarsan and mercury, as mentioned in the text.
Mercury alone	38	36	94·8	

In order to compare the effect of salvarsan with that of mercury in preventing the onset of secondary symptoms when treatment is commenced in the stage of the primary sore we have examined the notes on thirty-eight cases of primary sore which were treated with salvarsan, and compared their behaviour with that of thirty-eight similar cases which were treated with mercury only. The case-sheets of the latter were selected with two stipulations only: (1) that *S. pallidæ* were found in the sore and (2) that treatment commenced before the onset of any secondary signs. In each of the primary sores of the salvarsan series *S. pallidæ* were demonstrated in the sore previously to the commencement of treatment. All the cases were under observation for at least four months, and the minimum period of observation was in one of the two mercurial cases which did not show secondaries. Table II shows the result

of the scrutiny and demonstrates the superiority of salvarsan. It should also be mentioned that one of the two cases which developed secondaries in the salvarsan series first had a recurrence of the primary sore after transfer to another station, and was put on mercurial treatment, receiving a course of nine weekly injections, two months' rest and four fortnightly injections of the second course up to the date of onset of his secondary symptoms, which occurred twenty-five weeks after the sore returned; so that, strictly speaking, this is a case where mercury failed to prevent secondary signs.

The treatment of the mercurial cases was as follows: Of those which did not show secondaries, one had 9 weekly injections, a rest of two months, 3 injections of the second course and then passed to the Army Reserve; one had 10 weekly injections, 7 weeks' rest, 6 further injections, three months' rest, 4 more injections and then went to the Reserve; the remaining 36 cases which developed secondary symptoms did so as follows:—

4	after	the	1st	injection		2	after	the	6th injection
2	,,	,,	2nd	,,		1	,,	,,	7th ,,
4	,,	,,	3rd	,,		3	,,	,,	8th ,,
2	,,	,,	4th	,,		13	,,	,,	9th ,,
1	,,	,,	5th	,,		3	,,	,,	10th ,,
						1	,,	,,	11th ,,

The 3 cases which developed secondaries after the tenth injection had received 9 injections in the first course, then two months' rest, and the symptoms were noted immediately before the second injection in the second course. The last case had 11 injections in the first course. In no case did the first course consist of less than 9 injections. All were treated at Rochester Row.

The salvarsan cases were treated as follows: Of those which developed secondaries, 1 had an intramuscular injection of 0·6 grm., the sore recurred one month later, and he was put on mercurial injections as already detailed; secondary symptoms occurred twenty-five weeks later.

One had 2 intravenous injections of 0·4 and 0·5 grm. at an interval of two weeks and developed secondaries thirty-four weeks later.

Of those which did not develop secondaries, 3 had an intramuscular injection of 0·6 grm., and 1 of these was given an intravenous injection of 0·6 grm. for a positive Wassermann reaction twenty-six weeks later; all were under observation for at least nine months: 2 had an intravenous injection of 0·6 grm. and were under observation for at least nine months; 5 had 2

intravenous injections of 0·4 and 0·5 grm. at two weeks' interval, and all were under observation for at least nine months; 17 had an initial intravenous injection of 0·6 grm. followed by three intravenous injections of 0·2 to 0·3 grm. at fortnightly intervals; 6 of these were under observation for at least nine months; 3 for at least eight months; 6 for at least seven months; and 2 for at least six months; 9 had an initial intravenous injection of 0·6 grm., nine weekly injections of mercurial cream and, finally, an intravenous injection of 0·6 grm. salvarsan; 3 of these were under observation for at least six months; 4 for at least five months; and 2 for at least four months.

TABLE III.—To Illustrate the Effect of Mercurial Injections on the Wassermann Reaction in Syphilis.

Tested at end of	Original Method				Stern's Modification			
	Total cases	Positive	Negative	Per cent. positive	Total cases	Positive	Negative	Per cent. positive
1st course of 6-9 injections	89	66	23	74·1	39	32	7	82·0
1st interval of 6-8 weeks	21	15	6	71·4	14	11	3	78·5
2nd course of 4-6 injections	80	43	37	53·7	36	27	9	75·0
2nd interval of 2-3 months	27	19	8	70·3	24	23	1	91·6
3rd course of 4 injections	80	42	38	52·5	46	35	11	76·0
3rd interval of 2-3 months	34	18	16	52·9	24	18	6	75·0
4th course of 4 injections	121	43	78	35·5	90	53	37	58·8
4th interval of 4-6 months	41	24	17	58·5	27	17	10	62·9
5th course of 4 injections	65	23	42	35·3	45	28	17	62·2
5th interval of 4 weeks	60	16	44	25·1	44	26	18	59·0
6th or 7th course of 4 injections	105	39	66	37·1	71	47	24	66·1
Three months after end of usual period of treatment	170	67	103	39·4	105	80	25	76·1

The behaviour of the Wassermann reaction under mercurial and salvarsan treatment respectively is shown in Tables III and IV. No case is included in Table IV which received any treatment previously to the administration of salvarsan.

TABLE IV.—To Illustrate the Effect of Salvarsan on the Wassermann Reaction in Syphilis, the Patients having Received no Previous Treatment.

Tested at the end of	Original method				Stern's modification			
	Total cases	Positive	Negative	Per cent. positive	Total cases	Positive	Negative	Per cent. positive
3-4 months..	76	12	64	15·7	65	18	47	27·6
6 months ..	58	16	42	27·5	48	20	28	41·6
9 months ..	28	10	18	35·7	21	11	10	52·3

The great majority of the mercurial cases had received nine injections in the first course, so that the end of the first rest corresponds to three or four months from the commencement of treatment, six months with the end of the second course, and nine months with about the middle of the third course. The tables illustrate the more intense effect of salvarsan and show that the clinical results do not represent a mere cloaking of symptoms. At the same time we are quite alive to the fact that an important percentage of the salvarsan cases remained uncured at the end of three, six and nine months respectively. Though this may be accounted for to a certain extent by the fact that Table IV includes a number of cases treated by intramuscular injection, and we believe that better results will be shown by those treated on later plans, the percentage of positive reactions remaining at the end of the periods stated shows the necessity of including the Wassermann test in the subsequent observation of cases treated with salvarsan. The same remark applies equally to cases which are treated with mercury; in this connection, we would draw attention to the results which were obtained by the Wassermann test three months after the termination of the usual two years' mercurial treatment, averaging twenty-eight injections, and would take this opportunity of urging that when patients are treated with mercury alone the treatment should be more intense, especially in the early stages. Our plan is, at present, to arrange for cases which are treated with mercury alone to have at least forty injections in two years.

Regarding the actual administration of salvarsan, as we mentioned in a previous paper, we have discarded the subcutaneous and

intramuscular methods in favour of the intravenous. The sole disadvantage of the latter method is the reaction which sometimes follows the injection in a few hours, and we have tried many plans of eliminating this. It was at first attributed to the salvarsan, till we remembered that similar reactions may follow the intravenous infusion of plain saline. After varying the salt content of the infusion without success, we noticed Wechselmann's suggestion that the reaction was due to micro-organisms in the salt solution and distilled water. At first sight, this would appear to have little foundation, seeing that we always autoclaved our solutions at 130° C. for at least twenty minutes previously to using them for solution of the salvarsan. Wechselmann pointed out, however, that when the salt solution and distilled water have been prepared for some days micro-organisms grow in them and that injection of their dead bodies is sufficient to provoke the reaction. Acting on this, we commenced to make a practice of preparing the salt solution on the same morning as the injection, autoclaving it immediately after preparation, as we judged that bacteria would be more likely to grow in normal saline than in distilled water. Subsequently we began to distil the water on the same morning as the injection and still later we took Merck's purest sodium chloride into use. The results of these successive changes were as follows: Of 32 consecutive cases injected previously to introducing the changes mentioned, 8 had a rigor; 26 had a temperature of 100° F. or over; 17 vomited. When the salt solution was prepared on the morning of injection, of 35 consecutive cases, none had a rigor; 15 had a temperature of 100° F. or over; 9 vomited slightly. When, in addition, the water was distilled on the morning of injection, of 25 consecutive cases, none had a rigor; 6 had a temperature of 100° F. or over; 3 vomited a little and 2 had slight diarrhœa. When Merck's sodium chloride was taken into use, in addition to the above, of 35 consecutive cases, 1 had a rigor; 10 had a temperature of 100° F. or over; 4 vomited slightly and 3 had mild diarrhœa.

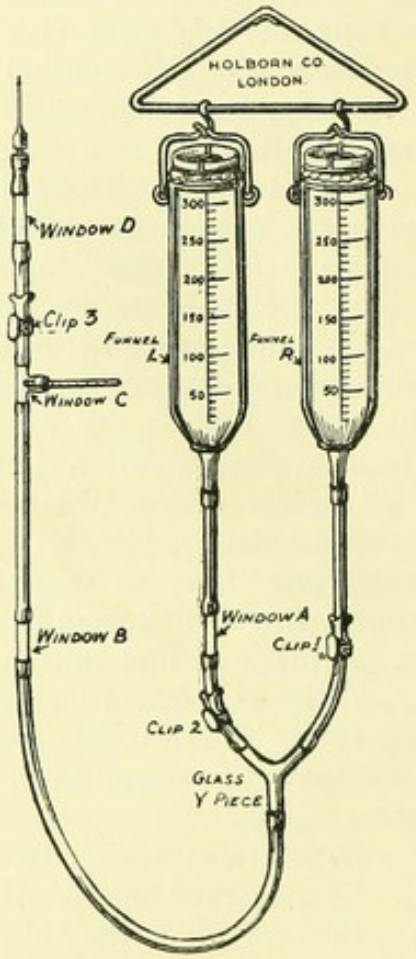
From this it is apparent that the preparation of the salt solution on the morning of injection had the greatest effect in reducing the number and severity of the reactions and that this was still further enhanced by the fresh distillation of the water, but that the use of Merck's sodium chloride had no advantage over that supplied as pure by the contractors. The patients, being in bed, do not notice a transient temperature below 102° F., so that subjective symptoms have been very mild since the changes were introduced.

It is not a matter of great difficulty to prepare the salt solution freshly, but we can easily understand that in hospitals not provided with a still which works rapidly it may not be easy to prepare the distilled water on the same morning. In these cases we would suggest that the water be distilled into Erlenmeyer flasks, which have been thoroughly cleansed previously, that the water be sterilized at once in the autoclave or steamer, or by boiling it for at least half an hour, and that then the neck of the flask be closed with a sterile rubber bung and tied over with sterile lint. When the only means of sterilizing the salt solution is by boiling it for thirty minutes, it is necessary to remember that this process concentrates the solution, and its volume must be made up to the original with sterile distilled water afterwards. The upper level of the solution should be marked on the outside of the flask ; therefore, before the solution is boiled, and this can be done more accurately on an Erlenmeyer than an ordinary spherical flask.

We have modified the apparatus described in this Journal of April, 1910, in a few respects and its present form is shown in the illustration. The glass Y-piece, at which the two rubber tubes (conveying salt solution and salvarsan respectively) meet, has been placed much nearer to the two funnels. Each of the latter has been fitted with a detachable strainer, which consists of two wire rings, a larger one above to fit outside the rim of the funnel and a smaller one below to fit inside it, the two rings being joined by wire stays. A piece of fine muslin is placed over the mouth of the funnel, and the wire frame then pressed into position, as shown in the illustration. The funnels are sterilized with the strainers in position, so there is no handling of strainer cloth afterwards. A thermometer to register the temperature of the solution just before it reaches the vein has also been introduced at window C. The latter is a glass T-piece, the stem of which is fitted with a perforated rubber bung through which the bulb of the thermometer is thrust. The thermometer should be kept in 1 in 1,000 perchloride of mercury when not in use and washed free of the chemical under a hot water tap immediately before being inserted in the T-piece.

To use the apparatus as it is now arranged, some salt solution is poured into both funnels, clips 1 and 2 being closed and clip 3 open. The tubing is then held by the needle end as high as possible above the level of the salt solution, and clips 1 and 2 opened. It is then slowly lowered till salt solution begins to flow from the needle end. Clip 1 is then closed, but clip 2 left open

till the solution in funnel L (which is intended for salvarsan) has nearly reached the bottom of the funnel. The needle should be fitted while the solution is flowing. Clips 2 and 3 are then closed and clip 1 opened. The dose of salvarsan solution is poured into funnel L, and enough salt solution into funnel R to reach the 100 cc. mark or over. The funnels are raised to a height of 4 ft.



above the level of the operating table, and all slack tubing coiled into a bowl containing water at about 120° F. Clip 3 should then be opened and the thermometer watched. The mercury will probably fluctuate rather widely at first, but will presently become steady at about 100° F. Clip 3 is then closed, and all is ready for the operation. When the needle has been introduced into the vein clip 3 is opened, and the solution in funnel R watched to see that it is flowing satisfactorily. As soon as this has been ascertained,

and it is seen that no swelling has formed round the vein, clip 1 is closed and clip 2 opened, and the salvarsan solution will flow steadily into the vein. It will be noticed that we do not now wait for blood to appear at the first glass window, but rely on the salt solution flowing satisfactorily, and the absence of any swelling round the vein, as indications that the needle has been properly introduced. In fact, we raise the funnels well above the operating table from the commencement in order to prevent blood from flowing back into the tubing. The blood gives no more information and might block the needle. It is easy to regulate the temperature of the solution during the administration by adding hotter water to that in the bowl in which the tubing is coiled, or removing some of the tubing from the hot water, according to whether the level of the mercury in the thermometer falls below or rises above 100° F. When the last of the salvarsan solution has passed clip 2 and just appears at the glass Y-piece, clip 2 is closed and clip 1 again opened. Salt solution is thus allowed to flow from funnel R till it has replaced the yellow salvarsan solution in window D. The needle is then withdrawn. The patient should rest in bed and be kept on a light diet till noon the following day.

We suggest the following scheme of treating syphilis with salvarsan from the commencement with the full consciousness that it may require some modification when sufficient time has elapsed to allow us to judge of its imperfections rather more fully than at present. We are well aware that some other workers, notably Gennerich, recommend a much more strenuous treatment, but while we aim at achieving the highest possible percentage of cures we desire to do so by the simplest method which is compatible with that end. So far we have obtained the most satisfactory results with the scheme of management which we are now advocating, and, as the great majority of Army cases are constantly under careful supervision, a scheme on more strenuous lines can be introduced later if the suggested scheme be found eventually to be insufficient, without any harm having happened to the patients. We therefore recommend the following:—

(1) No effort should be spared to make the diagnosis as early as possible. This is particularly important in the primary stage, and we cannot emphasize too strongly the importance of searching for the *Spirochæta pallida*, which can be found and the diagnosis established long before the sore is indurated. In fact, when induration has occurred our experience shows that the Wassermann reaction has already become positive in the great majority of cases,

and valuable time has been lost. The primary sore should be treated with vigorous local measures, excision when possible, and the use of the thermo-cautery or 30 per cent. calomel ointment in other cases.

(2) An injection of salvarsan should be given intravenously with the least delay, and the dose should be the largest which the patient is likely to stand. Almost every soldier can be given 0·6 grm. without fear. Some workers recommend that previously to the administration of salvarsan a course of mercury should be given. Considering the immensely superior effect of salvarsan in arresting the activity of the *S. pallida*, we can see no reason for such a course. In fact, we hold very strongly that the first remedy to administer should be the one which will at once deliver the most crushing blow to the parasite.

(3) For reasons we have mentioned above, it is always desirable to follow up the salvarsan with mercury, either in the form of intramuscular injection or *efficient* inunction. If the former be preferred the course should consist of nine weekly injections (Hg. gr. i in each), or three of calomel cream (Hg_2Cl_2 gr. $\frac{3}{4}$ in each) and six of mercurial cream.

(4) On completion of the course of mercury it is advisable to administer another injection of salvarsan, giving the same dose intravenously as in the first instance.

It is difficult at this stage to recommend a definite procedure as to subsequent observation. On the one hand, we have shown above by the fact that relapses occur in a certain proportion of cases and, in more instances, the Wassermann reaction remains positive, that not all cases treated by salvarsan are cured at once, so that it will certainly be necessary to keep patients under observation after completion of this combined course. On the other hand, we think that this observation need not require the patient's attendance so frequently as under treatment by mercury alone. In order to arrive at some conclusion as to the length of time it may be safe to excuse the patient's attendance for examination immediately after the completion of the combined course, we have examined the histories of 81 cases which had previously received no treatment whatever and were observed for three months after the last injection of salvarsan in the initial course, as detailed below, to ascertain the number which relapsed in this time. Of the 81 cases, 15 received an intramuscular injection of 0·6 grm.; 6 received an intravenous injection of 0·5-0·6 grm.; 20 received two intraveneous injections of 0·4-0·6 grm.; 34 received an intravenous injection of 0·5-0·6 grm., followed by 3

of 0·2-0·3 grm. at fortnightly intervals, and 6 received the combined course outlined above.

Three of the above cases relapsed within the three months: one after a single intramuscular injection and two after a single intravenous injection. In two of the relapses it was the primary sore which returned. On the strength of this, we think that it would be safe to excuse the patient further attendance for three months after completion of the combined course we recommend, particularly if he is told to report sick in the event of any recurrence of active signs. At the end of the three months it is highly important that the blood should be examined for the Wassermann reaction, and this blood examination should be repeated at three monthly intervals for the remainder of the two years from the date of first injection. If on this or any subsequent occasion the Wassermann reaction should be positive, the treatment should be repeated at once. Though a positive reaction does not always mean an imminent clinical relapse, yet in a high proportion of cases which relapse clinically the Wassermann reaction becomes positive some weeks previously, and, if we may judge from the effect of repeating the injection in cases which do relapse, there is good reason to believe that a repetition of the treatment as soon as the Wassermann reaction has been noted as positive will have the effect of averting a large number of clinical relapses. Apart, however, from the advantage of saving a certain amount of inefficiency through residence in hospital for relapses, our object must always be to obtain a permanent cure, and it is reasonable to suppose that this end will be more easily attained if we attack the parasite when it is only sufficiently active to provoke a Wassermann reaction, rather than wait till it has revived sufficiently to call forth outward signs of syphilis.

Regarding the intervals which should elapse between clinical examinations of the patient, we think that attendance every four weeks after the three months has elapsed from the completion of the combined course should be sufficient if the blood is examined as we have recommended. It must be remembered that this scheme applies only to cases which are treated from the first with salvarsan, not to those in which this remedy is given after a more or less extended trial of mercury; the latter would include a number of intractable cases of older standing, and such, being more prone to relapse, should be observed more frequently.

In view of the somewhat alarming reports which some authors have made on the occurrence of disorders of the nervous system

following the use of salvarsan, it is necessary that we should add our own experience in this matter. In one only of all our cases has any affection of the nervous system followed the administration of salvarsan, when no previous disease of the nervous system existed. In this case the patient was admitted for secondary ulceration of the tonsils, which had not yielded to calomel and mercurial cream injections, the primary sore having occurred twelve months previously. He was given an initial dose of 0·6 grm. salvarsan, followed by three intravenous injections of 0·2 grm. at fortnightly intervals. The throat healed promptly, but he developed symptoms of spinal meningitis two months after the last injection. These increased till he was re-admitted to hospital one month later. On re-admission he was also suffering from gonorrhœa. He recovered sufficiently to return to duty a month after this, but the symptoms subsequently returned, and he was eventually invalided three months later. There seems to us no more reason for attributing to salvarsan the symptoms which this patient developed subsequently to the injection, than for ascribing to the effect of mercury the ten cases of disease of the nervous system, excluding parasyphilis, which developed under mercurial treatment and were eventually treated by us with salvarsan. In these cases, complete relief resulted in six, and marked benefit in the remaining four. Finger states that 4 per cent of primary cases, 12 per cent of secondary, and 2 per cent of tertiary cases, which are treated with salvarsan subsequently develop affections of the nervous system in from two to eight months, and criticizes the published statistics on this subject, on the grounds of insufficient subsequent observation. We have treated 392 patients with salvarsan, including 66 primary, 281 secondary, 38 tertiary, and 7 parasyphilitic, and of all these,

216	have	been	under	observation for at least	4 months
190	,,	,,	,,	5	,,
166	,,	,,	,,	6	,,
142	,,	,,	,,	7	,,
114	,,	,,	,,	8	,,
94	,,	,,	,,	9	,,
66	,,	,,	,,	19	,,
49	,,	,,	,,	11	,,
35	,,	,,	,,	12	,,
19	,,	,,	,,	13	,,
11	,,	,,	,,	14	,,

176 having been observed for less than four months since the date

of the first injection; so that insufficient observation does not account for the freedom of our patients from these nervous affections. As regards the question of disasters immediately following the injection, we can only say that in 43 intramuscular and 791 intravenous injections administered up to the present date (December 6, 1911), we have had no death, and in one case only, a very early one, has any local trouble followed an intravenous injection. In this case some of the salvarsan solution had been inadvertently injected into the tissues on the deep side of the vein, and the arm was swollen for ten days afterwards. At the same time it is necessary to remark that in administering salvarsan intravenously it is essential that every detail of technique should be strictly observed. The fact that there is nothing in the technique which is beyond the capacity of anyone with ordinary skill and intelligence does not make salvarsan a remedy which can be administered carelessly with impunity.

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THE ANTI-BACTERICIDAL ACTION OF THE BILE SALTS.[1]

By Major S. LYLE CUMMINS.
Royal Army Medical Corps.

Bile and the bile salts are substances of great importance in connection with typhoid fever. On the one hand, they are extensively used in differential media for the isolation of *Bacillus typhosus* from the excreta, and in media designed to cultivate the bacillus from the blood; while, on the other, the survival of the organism in the gall-bladder and its association with gall stones and cholecystitis indicate that bile may play an important *rôle* in the etiology of the disease. It would appear, therefore, that a study of the mode of action of bile in culture media for the isolation of *B. typhosus* might, apart from its bearing on bacteriological technique, incidentally throw light on the far more important question of typhoid fever and the production of typhoid carriers.

The action of bile and the bile salts in favouring the growth of *B. typhosus* on differential media has been most ably investigated by Dunschmann,[2] but that observer did not extend his work to explaining the uses of bile in blood-culture, beyond the fact of its "enriching" action on the growth of the typhoid bacillus, and its power of retarding the growth of certain other organisms.

Eppenstein and Korte[3] called attention to the anti-bactericidal action of bile, and Conradi,[4] recording further experiments to the same effect, pointed to this anti-bactericidal action as explaining the utility of bile in the culture of typhoid bacilli from the blood. A feature of Conradi's work was that he found bile to inhibit the bactericidal properties of *serum*, a point to be borne in mind, as Gildmeister, in a recent communication,[5] attributes the use of bile in typhoid blood-culture entirely to its hæmolytic action, which he supposes to liberate anti-bactericidal substances from the disintegrated blood-cells.

Pies,[6] referring to Conradi's work above quoted, lays stress on

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[1] Reprinted by permission of the Editors of the *Journal of Hygiene*.
[2] *Ann. Inst. Pasteur*, January, 1909, p. 29.
[3] *Münch. med. Wochenschr.*, 1906, p. 1152.
[4] *Ibid.*, p. 1361.
[5] *Arb. a. d. Kaiserl. Gesundheitsamte*, February, 1910.
[6] *Arch. f. Hygiene*, 1907, lxii, p. 125.

the high concentration of nutrient matter in the serum as an important factor in the survival of typhoid bacilli.

Experiments carried out by the author in collaboration with Major C. C. Cumming, R.A.M.C.,[7] led us to the opinion that, in the isolation of typhoid bacilli from the blood, the anti-bactericidal action of the bile salts was of far greater importance than any enriching quality that they might possess. The experiments about to be recorded were undertaken with a view to elucidating as far as possible this anti-bactericidal action in the hope that some light might in this way be thrown on the survival of *B. typhosus* in "carriers," as well as on the properties of the bile salts as constituents of media for the culture of typhoid bacilli from the blood of cases.

Experiment 1. *Object.*—To confirm previous work in demonstrating the anti-bactericidal action of sodium taurocholate.

An agar slope of B. T. "Rawlings" was emulsified in 10 c.c. of sterile normal salt solution.

A series of dilutions of this emulsion were prepared of the following strengths :—

One in 1,000 ; 1 in 10,000 : 1 in 100,000 and 1 in 1,000,000. It was desired to compare the bactericidal effect of a 1 in 8 dilution of normal blood in sterile water with that of the same strength of blood diluted with a 0·5 per cent solution of sodium taurocholate. It was anticipated that on mixing known volumes of the blood preparations with equal volumes of successive dilutions of the typhoid emulsion, their relative bactericidal efficiency would be manifested in their power of sterilizing the bacterial dilutions in contact with them.

TABLE I.

		Dilution series, typhoid emulsion			
		1 in 1,000	1 in 10,000	1 in 100,000	1 in 1,000,000
A. Blood, 10 c.mm. Water, 60 c.mm. Dilution of emulsion, 10 c.mm.	Findings on plates	+	−	−	−
B. Blood, 10 c.mm. Taurocholate 0·5 per cent solution, 60 c.mm. Dilution of emulsion, 10 cmm.	Findings on plates	+	+	+	+

+ = growth, − = sterile.

Series A.—To 10 c.mm. of each bacterial dilution were added 10 c.mm. of freshly drawn blood and 60 c.mm. of sterile water.

[7] Journal of the Royal Army Medical Corps, June, 1910.

Series B.—To 10 c.mm. of each bacterial dilution were added 10 c.mm. of freshly drawn blood and 60 c.mm. of a 0·5 per cent solution of sodium taurocholate. The four preparations from each series were incubated at 37° C. for twenty hours, and then spread on plates. The result is shown in Table I.

It will be seen that the mixture of blood and water was able to sterilize a 1 in 10,000 dilution of typhoid emulsion; the presence of sodium taurocholate in a similar dilution of blood annulled all bactericidal effect on even so high a dilution of bacterial emulsion as 1 in 1,000,000.

This experiment has been frequently repeated and always with a like result. It might, however, be urged that the survival of the bacteria in contact with sodium taurocholate was due, not to any anti-bactericidal action of the salt but rather to the enriching power claimed for it by Dunschmann. To settle this point, it was decided to work out the enrichment power, if any, of the sample of sodium taurocholate under examination, leaving the action of blood out of the question.

Experiment 2.—In separate test-tubes were placed 10 c.c. of ordinary peptone and salt solution and 10 c.c. of a 0·5 per cent solution of sodium taurocholate in peptone and salt. To each tube was added 10 c.mm. of an emulsion of B. T. "Rawlings," and both preparations were incubated at 37° C. for three days. A "count" of each preparation was then made, with the result that the bile salt peptone water tube contained 235,000,000 bacilli in 1 c.c., while the peptone water contained 249,000,000 per 1 c.c.

It is obvious, therefore, that the sample of sodium taurocholate under examination has no marked enriching effect in three days—in fact rather the reverse.

It may then be taken as proved that sodium taurocholate is able to inhibit the bactericidal action of normal blood.

Since it is well known that the bactericidal efficiency of the blood-fluids increases during the process of clotting and is greater in the serum than in fresh blood, it next became a question whether the bile salt acted by preventing the elaboration of bactericidal substances during clotting or interfered with their activities after elaboration.

Experiment 3.—A broth-culture of B. T. "Rawlings" was diluted as in Expriment 1. A sample of blood was then withdrawn by finger-puncture, a portion of it, in 1 in 4 dilution, treated at once, and the remainder allowed to clot and the serum treated in the same dilution after ten minutes, two hours and six hours

respectively. All preparations were incubated for twenty hours and plated.

The result is shown in Table II.

TABLE II.

		Dilution series of typhoid broth culture			
		1 in 1,000	1 in 10,000	1 in 100,000	1 in 1,000,000
A. Fresh blood, 5 c.mm.; 0·5 per cent taurocholate solution, 10 c.mm.; Dilution of emulsion, 5 c.mm.	Findings on plates	+	+	+	+
B. As above but with "10 minutes" serum	,,	+	+	+	+
C. As above but with "2 hours" serum	,,	+	+	+	+
D. As above but with "6 hours" serum	,,	+	+	+	+

N.B.—The broth culture dilutions were kept at a temperature of 32° F. in the intervals of being used, to prevent multiplication of the bacilli.

This experiment showed that sodium taurocholate acted, not by interfering with the formation of bactericidal substances, but by inhibiting their action.

Before proceeding further in the mechanism of this inhibition, it seemed important to ascertain whether this power was shared by the glycocholate of soda also, and whether the constituents of these salts, taurin, glycin, and cholalic acid, were able equally to interfere with bactericidal action.

Experiment 4.—Dilutions of broth-culture of B. T. "Rawlings" were prepared as before.

Solutions containing 0·5 per cent of each of the above substances were made in sterile water, and a mixture of one part of fresh blood in three parts of each solution was then prepared.

To 10 c.mm. of each blood-mixture was added 5 c.mm. of each dilution of the typhoid culture, and the preparations incubated and plated as before.

The result is shown in Table III.

TABLE III.

			Dilution series of typhoid broth culture			
			1 in 1,000	1 in 10,000	1 in 100,000	1 in 1,000,000
A. Fresh blood 1 part; Sterile water 3 parts; Dilution of culture, 5 c.mm.	10 c.mm.	Findings on plates	+	–	–	–
B. Fresh blood 1 part; 0·5 per cent sol. of taurocholate of soda 3 parts; Dilution of culture, 5 c.mm.	10 c.mm.		+	+	+	+
C. As above, but 0·5 per cent taurin sol.		,,	–	–	–	–
D. As above, but 0·5 per cent glycocholate of soda sol.		,,	+	+	+	+
E. As above, but 0·5 per cent glycin sol.		,,	–	–	–	–
F. As above, but 0·5 per cent cholalic acid sol.		,,	+	–	–	–

It appears from the above that both sodium taurocholate and glycocholate possess anti-bactericidal qualities, while glycin, taurin and cholalic acid are without any such action, sterilization of the culture being active when blood is mixed with solutions of these substances.

The cholalic acid used was an old sample which had been long in the laboratory, and it will be desirable to test this acid further when a reliable preparation is available. It is curious that, in view of the proved absence of anti-bactericidal action in taurin and glycin, and the activity in this respect of the taurocholate and glycocholate of soda, the cholalic acid should be without this quality, but, assuming the sample used to be reliable, the above experiment certainly indicates that this is the case.

It is now time to return to the mechanism of this anti-bactericidal action of sodium taurocholate.

Regarding the disintegration of bacteria as a "complement-amboceptor" reaction, it may be assumed that bactericidal activity can be destroyed by preventing the action of amboceptor or of complement or both. The elucidation of this question is complicated by the difficulty of obtaining complement free from amboceptor, but this can to a certain extent be got over by comparing two sera of different bactericidal "titre."

Experiment 5.—*Object.* To ascertain whether sodium taurocholate interferes with the sensitization of typhoid bacilli by amboceptor.

The serum of a rabbit possessing a considerable degree of immunity to *B. typhosus* and agglutinating it in a dilution of 1 in 200, was heated for twenty-five minutes at 60° C. to inactivate its complement. The serum of a normal rabbit was obtained at the same time and treated in the same way. A twenty-four hours' agar culture of B. T. "Rawlings" was emulsified in saline.

The following mixtures were then prepared :—

(1)	Heated immune serum	1 part
	0·5 per cent sodium taurocholate solution	1 part
	Typhoid emulsion	2 parts
(2)	Heated normal serum	1 part
	0·5 per cent sodium taurocholate solution	1 part
	Typhoid emulsion	2 parts
(3)	Normal salt solution	2 parts
	Typhoid emulsion	2 parts
(4)	Normal salt solution	1 part
	0·5 per cent sodium taurocholate solution	1 part
	Typhoid emulsion	2 parts

These mixtures contained, in all cases, the same concentration

of typhoid emulsion, and, where it was present, of sodium taurocholate. They were left in contact, over night, at room temperature to ensure sensitization of the bacteria, if this indeed could take place in the presence of the taurocholate, in the serum preparations.

The mixtures were then centrifuged for forty-five minutes, the deposits washed in saline, and again centrifuged. After a final washing the deposits were emulsified in saline, the emulsions being "matched" by opacity to eliminate as far as possible fallacies arising through unequal multiplication and unequal "deposit"—the latter especially, as there was agglutination in *both* serum preparations, though of course this was more marked in the immune serum.

It was expected that if the bile salt had not interfered with the amboceptor in the heated immune serum, the bacteria in "mixture 1" would be so far sensitized as to be able to deflect complement to a greater extent than those placed in contact with normal serum. The saline emulsion was introduced as a "control" and the "bile salt—saline" mixture to make sure that the sodium taurocholate itself exerted no "sensitizing" action on the bacteria. The four emulsions were handed to Major L. W. Harrison, who very kindly undertook this part of the work. He examined them without knowing which emulsion was supposed to be the sensitized one, until the "key" was consulted after the experiment, and he reported as follows:—

Complete deviation occurred when emulsion No. 1 was placed in contact with 1 in 50 complement, partial when No. 2 was ditto, and none when Nos. 3 and 4 were placed under the same circumstances.

"From your key, it appears that bile salt did not affect the amboceptor. Possibly there was enough amboceptor in your normal serum, in the quantity used, to sensitize your bacteria; hence the partial result with No. 2."

The fact that there was agglutination with the "normal" serum would support Major Harrison's surmise as to the possibility of the presence of amboceptors.

The experiment goes far to prove that amboceptor is not interfered with by sodium taurocholate, and the anti-bactericidal action of this salt is therefore probably exerted through an inhibition of complement.

Experiment 6. *Object.*—To ascertain whether sodium taurocholate interferes with the action of complement.

The serum of the immunized rabbit used in Experiment 5 was heated for twenty minutes at 60° C. to destroy its complement.

Equal parts of this heated serum and an emulsion of B. T. "Rawlings" in saline were left in contact for one hour at 37° C. The sensitized bacilli were centrifuged, the deposit washed in saline, and the resulting emulsion diluted in series from 1 in 10 to 1 in 1,000,000.

A mixture of normal human blood one part and 0·5 per cent sodium taurocholate solution two parts, was then prepared and allowed to stand for one and a half hours. Ten c.mm. of this preparation was added to an equal volume of each dilution of the emulsion of sensitized cells. At the same time, as a control, 10 c.mm. of normal salt solution was mixed with an equal volume of each bacterial dilution.

Both series were then incubated for twenty hours and plated.

It was anticipated that, if complement were still active in the blood-bile salt mixture, this would enable the already sensitized bacilli to be dissolved, and the higher dilutions of bacterial emulsion would be sterilized.

On plating, however, it was found that there was complete growth in all the dilutions up to 1 in 1,000,000, proving that the blood, when mixed with sodium taurocholate, was unable to "complement" the sensitized bacilli.

It is evident then that the anti-bactericidal action of sodium taurocholate depends on interference with the complement, and not on inhibition of the action of the amboceptor.

It is not suggested that the taurocholate can prevent the complementing and digesting of sensitized typhoid bacilli when the latter have been ingested by phagocytes. Such observations as have been carried out indicate that phagocytosis and intracellular digestion of typhoid bacilli can both take place in contact with a 0·5 per cent solution of sodium taurocholate in citrated normal salt solution, though the destructive action of the bile salt upon the blood elements renders the observation difficult and unsatisfactory. But short of interfering with phagocytosis, the "anti-complement" action of the bile salts may perhaps have an important rôle in typhoid fever and the production of "carriers."

It may be permissible to consider for a moment the conditions obtaining in, say, the third week of an attack of typhoid fever. The agglutinating power of the blood is now high and the clumped bacteria have been, to a great extent, filtered out of the general circulation. It is tempting to imagine them "held up" in con-

siderable aggregations in the internal organs, such as the spleen, the liver and the adenoid tissue of the intestinal mucosa.

Probably the anchoring of "clumps" in these organs makes the work of phagocytosis both by leucocytes and tissue cells an easier task in some respects, but the ingestion of many virulent bacteria must also lead to the breaking down of leucocytes and, in all probability, the liberation of complement. In other words, at this time, there is probably an appreciable amount of extracellular solution of the typhoid bacilli: a surmise which is supported by the onset of the toxic symptoms characteristic of the later stages of the disease.

But while the liberation of complement leads, in most situations, to the extracellular solution of the already sensitized bacilli, the presence of bile at any given point would presumably prevent this solution, and enable even sensitized typhoid bacilli to survive and multiply. It is just in the positions where such an anti-bactericidal action of the bile is possible that foci of infection are found in typhoid carriers, *e.g.*, throughout the hepatic area and in the mucosa and walls of the gall-bladder.

The hypothesis put forward, while perhaps too speculative to be of value in itself, may give point to the experiments here recorded, and emphasize the importance of further work on the possible rôle of the bile salts in typhoid fever and its sequelæ.

In conclusion, I would express my indebtedness to Majors L. W. Harrison and C. C. Cumming, R.A.M.C., for their kind help, and to the editors of the *Journal of Hygiene* for several references to German literature which were of great service in compiling this paper.

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Original Communications.

CELL-INCLUSIONS IN THE BLOOD OF A CASE OF BLACKWATER FEVER.

BY LIEUTENANT-COLONEL SIR WILLIAM B. LEISHMAN, F.R.S.
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OUR knowledge of the ætiology of blackwater fever is still far from clear, in spite of the numerous laborious and careful researches which have been carried out in connexion with cases occurring in all parts of the Tropics; it appears therefore worth while to put on record the following observation. There is but slight ground for assuming that the bodies described below have any causative connexion with the disease, but others, more favourably situated as regards clinical material, may possibly be led to look for them and to follow up a line of investigation which might, conceivably, throw light on the dark places in our knowledge of this disease.

The material on which this note is founded is admittedly of the scantiest nature, as it consisted only of three unstained blood-films from a case of blackwater fever which occurred in Uganda, and which were sent to me by the Principal Medical Officer, Dr. A. D. P. Hodges, to whom I would here express my warm thanks. No clinical details were furnished, but such have been asked for and, if they should throw any useful light on the matter, will form the subject of a further note.

On first glancing at the stained films I was struck with an abnormal feature in the shape of the presence, in large numbers, of cells of an unusual type. These cells displayed considerable variations in shape and were of exceptional size, the average diameter being about 25 microns. They were of mononuclear type but differed

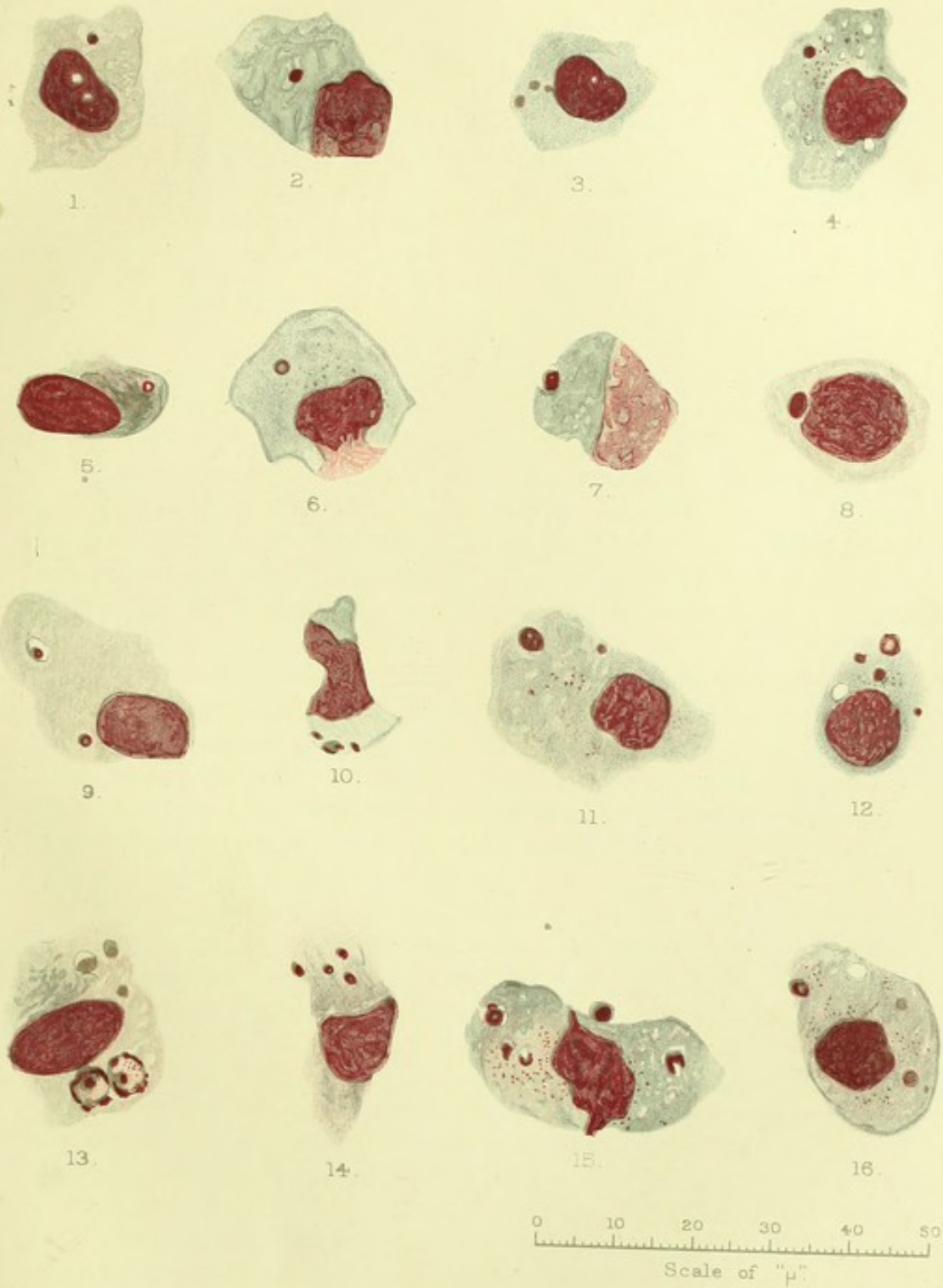
from the usual hyaline leucocytes, not only in their greater size but in the character and position of the nucleus and in the staining of the protoplasm. As regards the first of these points, the appearance of the nucleus, this but rarely showed any tendency to lateral indentation or to the horse-shoe appearance common in hyaline leucocytes, and, more often than not, it was situated excentrically and even pressed up against the side, while occasionally it extended completely across the cell. To save a more lengthy description a glance at the plate will show some of the types encountered and figs. 2, 4, 5, 6, 7, 9, 10, 14 and 15 demonstrate this particular feature. It is also noteworthy that the nuclear contours were always quite sharp and well defined, and that there was no evidence of any degenerative change of the nature of karyolysis or karyorrhexis ; the nuclei stained well, though in different degrees of depth according to the looseness or otherwise of the nuclear network.

The protoplasm of these cells stained a pale blue but did not exhibit the transparent appearance so distinctive of the hyaline leucocytes; it also frequently showed a reticular appearance more suggestive of the protoplasm of tissue-cells than that of leucocytes.

In the majority of instances the protoplasm was devoid of granules, but minute granules were found in a few, in very varying numbers and most unevenly distributed throughout the cell. As far as could be judged from the stain employed, that of the writer, these resembled the neutrophile granules of the ordinary polynuclear leucocyte or the neutrophile myelocyte, except that they seemed smaller and, in some cells, were of a pinker hue than the latter. The possibility of their being of another nature will be dealt with later.

I cannot speak with certainty as to the true nature and origin of these cells, but for various reasons I am inclined to regard them as endothelial cells which have been disrupted from the walls of the blood-vessels or lymphatics, and have been washed into the circulating blood. They appeared to resemble very closely the endothelial cells which are found free in the spleen, and in the capillaries of the liver in cases of kala-azar and which, in that disease, are more or less heavily infected with the characteristic parasites. I have not myself encountered them before in films of blood from blackwater cases, but similar cells have been noted and carefully described by Christophers and Bentley[1] in the blood of the cases which they

[1] S. R. Christophers and C. A. Bentley, "Blackwater Fever," Sci. Memoirs, Officers of Med. and San. Dept., Gov. of India. No. 35, 1908.



To illustrate "Cell-Inclusions in the Blood of a Case of Blackwater Fever."
By Lieut.-Col. Sir William B. Leishman, F.R.S., R.A.M.C.

investigated in India, and they, too, regard them as endothelial in origin.

No information is available as to the actual number of leucocytes present in the blood, but, to judge from the films, a moderate degree of leucocytosis must have been present.

The relative proportions of the various cells were as follows :—

Polynuclears	41·5 per cent.
Lymphocytes	6·0 ,,
Hyalines	4·5 ,,
Eosinophiles	0·5 ,,
Mast cells	0·5 ,,
Transitionals	8·5 ,,
Neutrophile myelocytes	2·5 ,,
Turk's cells	2·5 ,,
Endothelial (?) cells	33·5 ,,

A few normoblasts were found and, in one of the three films, an undoubted megaloblast. No striking changes were manifest in the red cells, which stained evenly and well and exhibited no evident deficiency of hæmoglobin ; basophilia was not seen and there were no poikilocytes or cells of exceptionally large or small size.

Careful search was made for malarial parasites, always a point of interest in view of the widely held views as to the connexion of malaria and blackwater, but none were found. At the same time, three or four pigmented leucocytes were found containing clumps of what appeared to be melanin, so it seems probable that parasites were either present or that the patient had very recently suffered from an attack of malaria.

Turning now to the cell-inclusions which are the chief feature of interest of this blood, these were altogether confined to the large endothelial cells. They were far from numerous, only about one cell out of twenty containing them, but they could hardly have been missed as they formed very clear and arresting features. It is not possible to say whether they would have been made manifest by the customary five minutes' application of the writer's stain, since each film was stained deeply by half an hour's contact with the mixed stain and water. By employing the stain in this fashion and subsequently washing the film with 60 per cent. alcohol, every trace of deposit left by the stain is dissolved off; this is essential for bringing out the details of fine structure and has the further advantage that we can be certain that we are not dealing with artefacts due to the deposition of stain.

In all cases the inclusions were seen to be contained in the

cell protoplasm, none were seen on or in the nuclei and only rarely were they seen in close contact with the nuclear membrane.

A lengthy description of the bodies may be dispensed with by referring to the coloured plate. The sixteen cells there depicted include more than half of the total number of cells in the three slides which were found to harbour inclusions. It will be seen that the inclusions range in size from a diameter of 1 to 5 microns, that they show a definite tendency to the circular in contour and that, while the majority present themselves under the aspect of ring forms, with deep-staining periphery and fainter hued centre, the smaller forms are homogeneous in their colouring. In all cases the inclusions took on a more or less pronounced pink or red colour, while many, as will be seen, showed the deep red reaction usually attributed to chromatin.

The two large inclusions seen in fig. 13 were remarkable in appearance and showed a dark-staining centre and an irregular but very deeply stained membrane or capsule; one other inclusion of the same type was found in another cell which is not figured as it was accidentally destroyed by a scratch before it could be sketched.

In several of the cells containing the inclusions well defined vacuoles of varying size were seen in the protoplasm, and since some of the smaller inclusions were found to be lying in similar clear areas, as for instance in figs. 9 and 13, it is not improbable that the empty vacuoles originally held similar bodies and that such vacuoles were not signs of degeneration of which indeed there was no other evidence in the cells in question.

The remaining feature of the sketches to which attention may be directed is the occurrence of fine granules in some of the cells; these have already been alluded to, and examples are afforded by figs. 4, 6, 11, 12, 15, and 16. It will be seen that they are extremely minute, but naturally it was not possible to depict them with any very precise regard to their real dimensions; they approached in many cases the limit of visibility, which is considered to approximate 0·1 micron.

The Possible Nature of the Cell-Inclusions.

It will be evident that these must fall under one of the following categories: (1) Artefacts; (2) products derived from changes in the nuclei or cytoplasm of the cells; (3) material which has been phagocyted by the cells; (4) micro-organisms. Each of these will be considered in turn.

(1) *Artefacts.*—The method of staining employed has already been mentioned, and it may safely be asserted that the inclusions were not derived from deposit or other change induced by the staining fluid. The writer's experience in connexion with this method enables him to speak with confidence on this point. Neither does it seem possible that the inclusions are artefacts due to fragments of foreign matter which had settled on or under the cells; the films were well made and free from dirt, and careful focusing showed that the bodies were actually inside the protoplasm of the cells, and were not lying either on or below them.

(2) *Products of Nuclear or Protoplasmic Change.*—It has been mentioned earlier that the nuclei of the cells appeared perfectly normal, and showed no signs of any degenerative change, such as karyolysis or karyorrhexis; their contours were always perfectly sharp. It seems, then, improbable they should have been extruded from the nuclei. At the same time it is possible that they may have had such an origin, in spite of the nuclei showing no direct evidence of this at the time the samples were taken. The appearance and staining reactions of the inclusions did not recall to me any products of nuclear change such as I have frequently encountered in other conditions. The possibility that they were products of changes, metabolic or other, in the cell cytoplasm also appeared unlikely, although this, too, cannot be absolutely excluded.

(3) *Phagocyted Material.*—The most likely objects to be taken into cells which are endowed with the property of phagocytosis are foreign particles of any sort, whether of organic or inorganic nature, and other cells. As regards the former the commonest material which is phagocyted in cases of chronic malarial infection, such as form the large majority of cases of blackwater fever, would be malarial pigment; as already mentioned, such pigment was found in leucocytes in this blood, but none in any other type of cell. Other extraneous matter was not in evidence outside the cells, and the inclusions noted in the endothelial cells appeared too regular in shape and structure to be regarded as of this nature. A more obvious explanation in this disease would be that the inclusions were only altered red cells, since phagocytosis of red cells is well known to be a striking feature in blackwater, although by no means limited to this disease. The most careful study of this feature of the disease, as far as I am aware, is that of Christophers and Bentley, already alluded to. These investigators record the

frequency with which the various cells of the blood and spleen exhibit the phenomenon and describe the various appearances which the ingested cells may assume. Among others, they describe the phagocytosis of red cells by the endothelial cells which are in question in this instance. Such cells they have found frequently in the blood during the acute stage of the disease, although the cells disappear rapidly when the hæmoglobinuria is passing off, and in many of them they observed evidences of phagocytosis of red corpuscles. Unfortunately their description was unaccompanied by plates or sketches, so it is not easy to contrast what they found with the cell-inclusions in this case, and I can only express my opinion that the inclusions are not to be accounted for in this manner. I am quite familiar with the appearance and staining reactions of phagocyted red cells in various stages of intra-cellular disintegration, but I have never observed them to take such an appearance as is to be seen in the accompanying plate.

A further observation is recorded by Christophers and Bentley, in which they noted in the blood of one of their cases on the fourth day of the disease a number of small mononuclear cells which had a deeply staining mass of nuclear-like substance lying within the protoplasm near the periphery of the cell; these bodies were small, averaging 1 to 2 microns in diameter, and they were inclined to regard them as nuclear extrusions. They also mention, in the case of large macrophages found in the spleen, that, among other inclusions such as red cells and pigment, some showed particles of a substance staining like chromatin, and somewhat resembling blood-plates. They had, however, observed these particles in other diseases than blackwater and were not inclined to attribute any special importance to them. Again, one may regret the absence of sketches, since it appears from the description that these latter bodies might perhaps have been similar to those found in the present instance. However that may be, the writer at all events has not observed them in specimens of splenic blood from any other disease.

(4) *Micro-organisms.*—Of these, bacteria may, I think, be safely excluded, at all events the inclusions do not bear the slightest resemblance to any known organisms of this nature. Blastomycetes might appear a more probable explanation, especially in connexion with the large forms shown in fig. 13, but the smaller bodies do not resemble any stage of the growth or multiplication of this class of micro-organisms.

As regards protozoa, at first sight these would appear to be even

more definitely excluded than bacteria were it not for the recent information which has come to light as to the probable life-history of the class of organisms to which Prowazek has given the name Chlamydozoa, and which he considers to be referable to the protozoa. The possible bearing of this information on the inclusions will be considered below.

The above short analysis of the various possibilities will at least show how numerous are the difficulties of ascertaining the true nature of the inclusions and how unjustifiable it would be to make any dogmatic assertions as to their origin. It appears to me, however, as at least possible that the inclusions in question may be due to the presence within these endothelial cells of Chlamydozoa. It need hardly be added that, even if this should prove correct, it would be a very long step between that knowledge and the proof that there was any causal relationship between these organisms and blackwater fever. Be that as it may, it would at least be an observation of considerab e interest that organisms of this class should be present in this mysterious disease.

Since knowledge relating to the Chlamydozoa is not yet widely diffused—indeed the name itself is probably unknown to many—it may not be inappropriate to include in this note a brief outline of the present state of the subject in order that the reasons for my suggestion may be more readily followed. Apart from this, much of the work in question suggests developments of the greatest importance in the near future in connexion with the causation of some of the most dangerous diseases of man, and has thus an interest of its own.

The organisms to which this name was applied by Prowazek, in 1907, have in most instances two features in common: they are capable of passing through the usual bacterial filter candles, at all events such as are fine enough in their grain to keep back the smallest known bacteria, and, second, in the diseases in which they occur "cell-inclusions" have been noted as a constant feature. Prowazek's original list included the following diseases: small-pox and vaccinia, rabies, trachoma, molluscum contagiosum, contagious epithelioma of birds, foot-and-mouth disease, and certain diseases of fish, dogs and silkworms. Since then Prowazek has modified this list and, according to Hartmann, who gives a good summary of the subject,[1] would only definitely include variola and vaccinia, trachoma, molluscum contagiosum and bird epithelioma. Evidence

[1] HARTMANN: *Beilage. z. Centr. f. Bakt.—Referate*, vol. xlvii., p. 94, 1910.

about the remaining ones he considers too incomplete to permit of their being retained in the same class at the present moment. In addition, however, to the above list it appears far from improbable that such diseases as scarlet fever, measles, chicken-pox, rabies and perhaps yellow fever may eventually be shown to be due to viruses of a similar class.

The longest known of the cell-inclusions are those found in molluscum contagiosum, but those associated with variola and rabies are more widely known and are, respectively, named after their discoverers, "Guarnieri's bodies" and "Negri bodies." When first found and described they were taken to be the actual parasites of the respective diseases in which they occurred, and various generic and specific names were attached to them, which it is unnecessary to recapitulate. Although demanding in most cases special staining methods for their demonstration, these inclusions were not difficult to find and recognize and, in spite of some reports to the contrary, it was soon recognized that they were specific to the diseases in which they were found. At the same time, there was much that was puzzling in their morphology and in their distribution in the tissue-cells; for instance, Negri bodies were chiefly found in the grey matter of the cornu Ammonis and the cerebrum and in Pürkinje's cells of the cerebellum, while they were found to be scanty or absent in other parts of the nervous system which experiment had shown to contain the virus of rabies in concentrated form. Again, Negri bodies are constantly found in rabic animals, in what is known as the "virus des rues," while they are as constantly absent in the nervous system of animals infected with "virus fixe." Another fact which seemed inexplicable in view of the large size of the inclusions was that, in practically all the diseases enumerated above, the virus has been shown to be capable of passing through very fine filter candles, the filtrate, cell and germ free, proving as virulent for animals as the unfiltered tissue products.

The non-parasitic nature of the inclusions appeared to be finally established when it was shown that Guarnieri's bodies of variola were soluble in strong saline solutions, and were broken up by both peptic and tryptic digestion. The inclusions then came to be looked upon as mere products of cellular reaction in response to the influence of the still unseen and unknown virus. They were, however, still looked upon as specific in the sense that they were only to be found in the particular disease, and might therefore have diagnostic value. As a matter of fact, in some Pasteur Institutes, it is now the custom to search the tissues

of a suspected rabid animal for Negri bodies, and to give a positive diagnosis if these are found; animal inoculation being only resorted to in the event of this examination proving negative.

A fresh impetus, however, has been given to their study by the discovery of very minute granules in some of the diseases in question, more particularly in trachoma, variola, and molluscum contagiosum, in association with the cell inclusions. More recently a similar association has been found in contagious epithelioma of birds, in rabies, in varicella and in both human and experimental scarlatina. The granules in question are extraordinarily minute, and many approach the limit of visibility, which, as has been said, postulates an object of 0·1 micron in diameter. Modern methods of staining, improved lenses and new methods of illumination have permitted the recognition of these minute particles either in the fresh state, where they are best observed by dark-ground illumination, and are seen to have very active oscillatory movements, or in dried films or sections where they are brought out by special methods of staining, of which some variety of Romanowsky is most frequently employed. Much of the work which has led to these results was carried out by inoculating the virus into the cornea of rabbits, where the subsequent appearance of both inclusions and granules has been observed and studied.

The most weighty evidence, however, as to the nature of the granules comes from the investigations of Prowazek and Aragão during a small-pox epidemic at Rio de Janeiro.[1] They found the granules were capable of passing through a Berkefeld filter, and that the sterile filtrate was still virulent, but on filtering the same material through a special filter coated with agar, an "ultra-filter" as they term it, the granules were retained and the filtrate found to be no longer virulent. On examining the surface of the ultra-filter great numbers of the granules were found, while the filtrate contained none, a marked contrast to what had occurred in the case of filtration through the Berkefeld candle, where the granules were as abundant in the filtrate as in the diluted lymph before filtration.

These and other observations which are constantly being reported appear to lead more and more to the conclusion that these minute microscopic granules are the veritable causes of the diseases in question. Owing to their minute size it is impossible

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1 S. von Prowazek and H. de B. Aragão. "Variola-unter suchungen," *Memorias do Inst., Oswaldo Cruz.*, tom. i., fasc. 2, p. 147, 1909.

to ascertain accurately their life-history, or to be certain whether they should be included among the bacteria or, as Prowazek suggests, among the protozoa, but what appears to occur is something of the following nature. A small granule gains entrance to a cell—for example, a conjunctival cell in trachoma, a nerve cell in rabies, or an epidermal cell in small-pox—and causes a reaction of the cell which is expressed by the throwing out of some reaction product in the shape of a capsule or mantle of secretion which surrounds the invading particle. (Hence the name Chlamydozoa, which is framed on the word, χλαμὺς, a cloak or mantle.) In some instances this covering mantle attains a very considerable thickness, and the body is conspicuous as the cell-inclusion known as a Guarnieri's body, a Negri body, a molluscum body, and so forth. The original granule, which may or may not be visible within the enveloping mantle, has been called the "initial body." The initial body then proceeds to divide, and from it are formed great numbers of the extraordinarily minute little particles which may eventually escape from the inclusion and fill the cytoplasm of the cell; these derivatives of the initial body are known as "elementary bodies," and it is held that it is in this form that the virus extends to other cells or to fresh hosts and that, by reason of their minute size, they are able to pass through filters, as has been described.

It will be seen, therefore, that in this view the recently discredited cell-inclusion is to be regarded as an evidence of the reaction of the cell to the true virus, the chlamydozoon granule, and that it acts as an enveloping cover or capsule to the latter which multiplies within it, forming the elementary bodies which are capable of transmitting the disease further afield.

Turning once more to the cell inclusions found in this case of blackwater fever, it is with the utmost reserve that I suggest that they, too, may bear the same relationship to a minute parasite of the nature of a Chlamydozoon and that there is no insuperable objection to the theory that such a parasite may prove to be the cause of blackwater fever. The total number of inclusions found in these three films is far too small to permit of any definite views being advanced as to a cyclical development, such as has been described above, but it is possible that the minute granules depicted in some of the cells may bear a relation to the inclusions similar to that which appears to obtain in the case of small-pox, rabies and molluscum contagiosum.

Assuming that those Chlamydozoa which have already been

described are the actual causes of the diseases with which they are associated, it will be noticed at once that from the clinical point of view there is little in common between these diseases, while, if we include among them the other diseases which may be suspected of having a similar kind of virus, such as scarlet fever, foot-and-mouth disease, measles and perhaps yellow fever, the clinical dissimilarity appears even more striking. Objections therefore founded on such dissimilarity between the above diseases and blackwater fever would not have much theoretical weight. Certain facts, however, which appear to be well established from the accumulated experience of blackwater fever, may be briefly considered in the light of the tentative suggestion which I have put forward. The majority of the diseases attributed to the Chlamydozoa are known to be infectious, and they frequently spread in epidemic form; epidemicity is certainly rare in the case of blackwater fever, if it occurs at all; at the same time, instances of the apparent epidemicity of the disease have been recorded, although the evidence in connexion with these has not been very complete. The question of infectivity would, on closer inspection, also appear not to be an insuperable difficulty when it is realized that in the large preponderance of the diseases mentioned the virus is obviously localized to a large extent in the skin lesions or in the secretion of mucous membranes; in blackwater, on the other hand, no superficial lesions are in evidence, and the virus, if a specific one exists, is probably situated more deeply in the organs or tissues. The mass of evidence which goes to show that blackwater fever is only contracted by those who are the subject of frequently repeated malarial infection and is practically confined to those districts which are known to be intensely malarious might, with as great propriety, be urged as a reason for suspecting that a specific virus may be transmitted by the bites of mosquitoes, or other insects, as in support of the view that the disease is largely attributable to chronic malarial infection. In this connexion the parallel of yellow fever might be adduced, as it is apparently the case here that a mosquito is the transmitter of a filter-passer, while a similar example might be quoted in the case of the still undiscovered filter-passing virus of pappataci or three-day fever.

Such speculations, however attractive, are of little value in comparison with positive evidence, and I will only put forward one more, which appears to me to count against the theory of a Chlamydozoon being responsible for blackwater fever. In the

majority of the diseases mentioned as being apparently due to these parasites an attack if recovered from confers a considerable immunity against subsequent infection; in blackwater, on the other hand, this is known not to be the case, as second and third attacks are not infrequent and may prove fatal.

Where all is indefinite it would appear out of place to end this note with the customary "conclusions"—these are still to seek. The main object has been to call attention to these bodies in the hope that further search may demonstrate whether there is any foundation for my suspicion—it is little more—that they represent an invasion of the endothelial cells of the visceral blood or lymph vessels by parasites of the nature of Chlamydozoa.

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NOTE ON THE BACTERIOLOGICAL EXAMINATION OF INDIAN WATER SUPPLIES.

By Major R. W. CLEMENTS.
Royal Army Medical Corps.

As the Sanitary Officer of the 9th (Secunderabad) Division, I have, since November, 1910, attempted to make definite bacteriological examinations of water samples within the command. These examinations have been based mainly on the instructions contained in Memorandum No. 4,555, drawn up by the Sanitary Officer at Army headquarters, and issued by the Principal Medical Officer, His Majesty's Forces in India, under date of September 21, 1910.[1] The results of these examinations are kept in a special book as a reference for the formulation of possible local standards. As it would involve too much space and expense to print in detail the report on each particular water sample examined, the main results of twenty-nine samples have been summarized in the accompanying table. It is notable to be able to record that on no occasion was a *Bacillus coli* of Escherich isolated from a drinking-water supply in this division. It will be admitted by most that this is a remarkable fact, and emphasizes clearly not only the need to examine critically Indian water samples bacteriologically, but also the necessity of revising accepted European standards.

It may be stated at the outset that the form of report adopted has been that drawn up by Major Clemesha, I.M.S., and published in his report on the bacteriology of drinking-water supplies in tropical climates.[2] Further, the classification of organisms isolated has been made on Clemesha's lines, or according to their power or resistance to sunlight, and the verdict as to recent or remote contamination with fæcal micro-organisms based on the class to which the micro-organisms isolated belong.

In view of the fact that the majority of water supplies in the 9th Division are wells or large tanks or ponds, and that storage in either form is known to have a powerful effect in reducing the number of bacteria present, it is not surprising that this factor, together with the action of sunlight in tropical climates, should

[1] Republished in the Journal of the Royal Army Medical Corps, November, 1910.

[2] Published by Government of India as Appendix I to "Annual Report of Kin Institute, Madras," 1908.

contribute largely to the purification of water in India, provided always that the supplies and storage reservoirs are properly protected. Heavy rains interfere with storage, and in addition we have the risk of surface contamination in both wells and tanks to consider. The possibility of fæcal material from both men and animals gaining access to the water under conditions of heavy rainfall is obvious.

The question of possible surface pollution of water supplies during heavy rain has been much neglected in India, and it is only slowly that faulty conditions are being remedied. Some wells in this division are now properly steined, covered in and provided with a pump to preclude the risks associated with the primitive and too prevalent system of hand haulage. By attention to this point several supplies, which were reported during 1910 as being dangerous or suspicious, have in 1911 been readily passed as safe. Another prevalent source of danger to our wells is the failure to guard carefully the adjacent area. A well drains an area of ground roughly about four times its own depth—more in a porous soil—and great care is required to see that this area is kept clean. To illustrate this, the water from a well in the officers' mess compound at St. Thomas' Mount has frequently been condemned as unfit for drinking. I inspected the well and found it surrounded by a small garden and flower-pots; it was at once evident that the source of contamination was manure from the garden and flower-pots. In most cases where wells yield contaminated water, this is due to faulty construction, and especially as regards steining. For example, if a well is 30 ft. deep, the sides for at least 20 ft. should be imperviously steined with bricks or stones, and lined with hydraulic cement. If this is done, water percolating from the surface must pass through at least 20 ft. of soil before entering the well, and in its passage through the soil, if the surroundings are protected, a large amount of organic impurity will be removed. As another example of imperfect steining the following may be quoted: depth of well 22 ft., the top 6 ft. steined with brick and mortar, and the lower 16 ft. with brick and mud. Such a well is liable to surface contamination unless the surroundings are carefully looked after. In this Division, Madras, St. Thomas' Mount, Poonamallee, Bellary, Calicut, Cannanore, and Mallapuram derive their water supply from wells, and in the event of any further improvements being carried out in connexion with these wells it is necessary that the question of steining with hydraulic cement be considered and adopted. The excellent diagram in Notter and

Firth's "Theory and Practice of Hygiene," third edition, 1908, p. 45, might well be taken as a guide for this kind of work by our military and public works engineers.

As a corollary to this question of the bacterial contents of Indian waters, much work has been devoted in our divisional laboratory to the study of the bacterial flora resulting in a water when contaminated with the excreta of various animals. The results are given below. They are both interesting and instructive, as throwing a side-light on the nature of the micro-organisms we so constantly find in Indian water supplies :—

(1) One gramme of cow dung rubbed up with half a litre of tap water. Of ten micro-organisms isolated, five were *Bacillus grünthal*, one Clemesha's No. 7, one *B. coli mutabilis*, and three *B. vesiculosus*. All belong to the class most resistant to sunlight, or Clemesha's Class III.

(2) As above, but water allowed to stand a day. All the micro-organisms isolated were *B. vesiculosus*.

(3) One gramme of rabbit dung rubbed up with half a litre of tap water. Six colonies were *B. grünthal*, and four were Clemesha's No. 106.

(4) Similar to above, but kept at room temperature for a week. Result of ten colonies isolated showed four *B. grünthal*, five No. 106, and one *B. neapolitanus*.

(5) As in four, but kept for a fortnight. Result showed, of ten isolated colonies, four to be *B. neapolitanus*, five No. 106, and one No. 71.

(6) As above, but after standing three weeks. All the colonies were either Clemesha's No. 71 or No. 106 ; that is, belonging to Class II, or those intermediate in their power of survival.

(7) One gramme of chicken droppings rubbed up with half a litre of tap water. Of ten colonies isolated, one was *B. coli communis*, seven were *B. grünthal*, and one each of Nos. 1 and 71.

(8) As above, but kept for a week. One was *B. coli*, one *B. neapolitanus*, one *B. grünthal*, one *B. coscoroba*, one No. 66, three No. 1, one No. 7, and one No. 71.

(9) As above, but kept for two weeks. Result : three *B. grünthal*, three No. 1, and one each of Nos. 7, 71, 74, and *B. coscoroba*.

(10) As above, but kept for three weeks. Result : of ten colonies isolated five were No. 106, two No. 1, two *B. grünthal*, and one No. 7.

(11) As above, but kept for a month. All the colonies isolated were those of Clemesha's No. 7.

(12) One gramme of horse dung rubbed up with half a litre of tap water. Result gave five of No. 1, two of *B. grünthal*, and one each of *B. coli*, No. 71, and No. 106.

(13) As above, but kept for a week. Result: of ten colonies taken four were No. 1, two No. 102, one *B. coli*, one *B. grünthal*, one No. 71 and one No. 106.

(14) Same as above, but kept for two weeks. Of ten colonies taken, three were *B. coli*, three No. 71, two No. 1, one *B. grünthal* and one No. 109.

(15) One gramme of dog's fæces rubbed up with half a litre of tap water. Result: of ten colonies six were *B. grünthal*, two *B. coli*, one No. 1 and one No. 36.

(16) As above, but kept for a week. Of ten colonies taken, four were *B. coli*, four *B. grnthal*, and two No. 106.

Note by Colonel R. H. Firth.

The foregoing came through my hands as part of the Annual Report of the Sanitary Officer of the 9th Division. As the writer of Memorandum No. 4,555 referred to, the subject matter naturally appealed to me, and in view of its general interest I am responsible for sending it to our Journal. I am also responsible for the particular form in which the tabular statement has been presented. In their original form the tables submitted by Major Clements did not lend themselves readily for publication.

That such an inquiry as this has been systematically attempted in the 9th Division is particularly encouraging, but it is matter for regret that other Divisional Sanitary Officers have not been able to initiate for their own areas an inquiry on similar lines. We have available, from Clemesha's work, valuable information as to the bacterial flora of Madras waters generally, and this record by Clements is confirmative of the published facts. It is evident that in the waters of Southern India, certain groups of lactose fractors are dominant. What we want to know is, how far the same groups and species are present in the ordinary water supplies of other districts, more especially in the Punjab, the United Provinces, and the western parts of India. The bacteriological examination of drinking waters is notoriously difficult even in Europe; the difficulties and fallacies associated with such work in India are infinitely greater. Under these circumstances, we need to be cautious how we interpret the results; but we shall never be any nearer the attainment of a position in or from which we can speak

Source of the sample	Date of examination	Total micro-organisms on agar at 37° per c.c.	Minimum volume in c.c. in which fæcal lactose fractors were present	Lactose fractors isolated and identified by subculture from ten selected colonies	Remarks
Secunderabad pipe supply (Jitmulla tank)	1. 6.11	580	0·1	*B. coscoroba* only	All Class II .. Safe.
Secunderabad pipe supply (Gun Rock wells)	20.11.11	Uncountable	0·1	*B. grünthal* (8), *B. coscoroba* (1), *B.* No. 73 (1)	Class II and III .. Safe.
Secunderabad pipe supply (Jitmulla tank)	21.11.11	Uncountable	0·1	*B. neapolitanus* only	Class II .. .. Safe.
Ditto (drawn from stand-pipe in R.A. lines)	3.12.11	60	25·0	*B. coscoroba* only	Class II .. .. Safe.
Secunderabad pipe supply (stand-pipe in 7th D.G. lines)	3.12.11	Uncountable	40·0	*B. cloacæ* only	Class III .. .. Safe.
Bangalore (after sedimentation but before filtration)	27.11.11	90	5·0	*B. lactis aerogenes* (6), *B. coscoroba* (2), *B. cloacæ* (1), and *B. grünthal* (1)	Class II and III .. Safe.
Ditto	26.10.11	110	25·0	*B. coscoroba* (5), *B. grünthal* (2), *B. cloacæ* (1), *B. lactis aerogenes* (1), *B. neapolitanus* (1)	Class II and III .. Safe.
Bellary. Artesian well	1.4.11	500	1·0	*B. grünthal* (5), *B. coscoroba* (4), *B. lactis aerogenes* (1)	Class II and III .. Safe.
Bellary. No. 1 well	17.7.11	850	0·1	*B. coscoroba* (8), *B. grünthal* (1), *B. neapolitanus* (1)	Class II and III .. Safe.
Bellary. Artesian well	27.7.11	740	0·1	*B. lactis aerogenes* (5), *B. cloacæ* (4), *B. neapolitanus* (1)	Class II and III .. Safe.
Bellary. No. 1 well	29.11.11	370	1·0	*B. cloacæ* (7), *B. neapolitanus* (1), *B.* No. 100 (2)	Class II and III .. Safe.
Wellington. Laboratory tap	4.3.11	460	5·0	*B. acidi lactici* (8), *B. cloacæ* (2) ..	Class II and III .. Safe.
Ditto	4.12.11	30	5·0	*B. grünthal* (4), *B. cloacæ* (6) ..	Class III .. .. Safe.

St. Thomas' Mount. Well in officers' mess compound	24.4.11	1,400	0·1	*B. lactis aerogenes* (7), *B.* No. 67 (3)	Class III	Safe.
St. Thomas' Mount. Well No. 16 ..	24.4.11	6,400	0·1	*B.* No. 105 (5), *B.* No. 97 (1), *B.* No. 98 (1), *B.* No. 99 (2), and *B. gasoformans non-liquefaciens*	Class I and II ..	Doubtful.
St. Thomas' Mount. Well in B.I. lines	24.4.11	1,300	0·1	*B. oxytocus perniciosus* (1), *B. rhinoscleroma* (3), *B. cloacæ* (4), *B. acidi lactici* (1), *B.* No. 105 (1)	Class I, II, and III	Doubtful.
St. Thomas' Mount. Well in N.I. lines	3.11.11	3,310	0·1	*B. cloacæ* (4), *B.* No. 6 (4), *B. neapolitanus* (2)	Class II and III ..	Safe.
St. Thomas' Mount. Well in R.A. lines	3.11.11	7,170	0·1	*B. lactis aerogenes* (7), *B. coscoroba* (2), *B.* No. 67 (1)	All Class II ..	Safe.
St. Thomas' Mount. Well in mess ..	3.11.11	3,160	0·1	*B. coli mutabilis* (3), *B.* No. 36 (4), *B.* No. 67 (2), *B. lactis aerogenes* (1)	Class I, II, and III	Doubtful.
Poonamallee. Well No. 16	15. 6.11	1,650	1·0	*B. grünthal only*	Class III	Safe.
Poonamallee. Well No. 13	15. 6.11	1,690	0·1	*B. neapolitanus* (5), *B. cloacæ* (4), *B.* No. 100 (1)	Class II and III ..	Safe.
Poonamallee. Well No. 13	14.11.11	890	0·1	*B. lactis aerogenes* only	Class II	Safe.
Poonamallee. Well No. 10	14.11.11	Uncountable	0·1	*B. coscoroba* (4), *B. grünthal* (3), *B.* No. 1 (3)	Class II and III ..	Safe.
Cannanore. Well No. 44 B	16. 3 11	430	25·0	All *B.* No. 109	Class III	Safe.
Cannanore. Well No. 47 D	7. 6.11	380	5·0	*B. grünthal* (8), *B. coli mutabilis* (2)	Class I and III ..	Doubtful.
Cannanore. Well No. 44 B	4.11.11	2,170	5·0	*B.* No. 109 (8), *B.* No. 1 (1), *B. lactis aerogenes* (1)	Class II and III ..	Safe.
Cannanore. Well No. 47 D	4.11.11	760	1·0	All *B. lactis aerogenes*	Class II	Safe.
Trichinopoli pipe supply	3. 1.10	360	5·0	*B. vesiculosus* (8), *B. coli mutabilis* (1), *B. coscoroba* (1)	Class II and III ..	Safe.
Ditto	14.10.10	6,220	10·0	*B. coscoroba* (7), *B.* No. 106 (2), *B.* No. 114 (1)	Class II	Safe.

with confidence, until more facts, and covering a greater area, are obtained and published. It is for the appreciation of this aspect of the question that I appeal.

Further experience, and the discussion of this matter with practical workers, leads me to doubt whether the presence or absence of lactose fractors only in an arbitrary volume of water, constitutes the best or more practical criterion by which we, in India, can attempt to appraise the quality of any particular sample which comes under examination. Everything points to the fact that in India lactose fractors are present in very small volumes of water which, from epidemiological and everyday experience, are undoubtedly safe waters, or at least waters incapable of causing disease usually associated with a fæcal origin. The total count per c.c. on agar is an equally doubtful datum, unless made always at once on collection of the sample. Too frequently, this is impossible to carry out. If this be so, it is obvious that we run great risks of condemning waters on unsound data, with every chance of bringing the whole procedure of water bacteriological examinations into disrepute. The pressing need, apart from reliable data, is for a reasonably simple and rapid technique or procedure, by which a busy man can form a reasonably sound opinion as to danger or safety in the case of samples submitted. We must not forget that there are times when men receive as many as from twenty to forty samples of water for examination within a few days. What is a Sanitary Officer to do under such circumstances? I was present, recently, at such a sequence of events. Each sample was put through the lactose fractor reaction, and five-sevenths of the samples, on suggested standards, fell into the condemned category. The situation was ludicrous. A personal visit to eight out of ten of the sources from which the incriminated samples had been taken showed that the local conditions pertaining both as to the place and health of the users of the waters warranted no condemnation. The question is, what can we do towards arriving at a sound working procedure, likely to give us the best and safest results. I confess I hardly know.

To meet the needs of routine work and of the busy man confronted with many samples, I suggest the following tentative procedure: Prepare and keep four stock solutions, each marked respectively as LA, SA, AA, and DA. These stock solutions to have each and all the common composition of peptone 60 grm., sodium taurocholate 15 grm. to one litre of water, with 10 c.c. of a 5 per cent solution of neutral red added, and the whole standardized

to the neutral point. The one marked LA must in addition contain 15 grm. of lactose, the one marked SA must contain 15 grm. of saccharose, the one marked AA must contain 15 grm. of adonit, and the one marked DA must contain 15 grm. of dulcit. Take twelve test tubes, each containing a fermentation tube, and in three of these place 5 c.c. of stock LA, in three place 5 c.c. of stock SA, in three place 5 c.c. of stock AA, and in three place 5 c.c. of the stock DA. Mark each set carefully, and to each of the three tubes in each set add respectively 5 c.c., 1 c.c. and 0·1 c.c. of the water under examination. Incubate for twenty-four hours at 42° C. or for thirty-six hours at 37° C. If all the series show acid and gas it is practically certain the water is contaminated by fæcal bacilli. If two out of three of each set show acid and gas, there is strong presumptive evidence of fouling. The critical information is to be drawn from the fermentation reaction in the respective three sets containing saccharose, adonit and dulcit. Thus, assuming we get a positive fermentation reaction in all these three and in the lactose or LA as well, the inference is justifiable that the water contains bacilli associated with recent fouling, or those which are of low resisting power. The type of this group of bacilli is the *Oxytocus perniciosus*. If there is fermentation in LA and DA only, it is probable that subculturing will show the pollution to be mainly by *B. coli communis*. If fermentation is evident only in LA and SA, the water probably is of the reasonably safe type and containing either *B. coscoroba* or *B. cloacæ*, or both. If there is marked fermentation only in LA and less marked in SA and AA and none in DA, the probabilities point to *B. lactis aerogenes* as a dominant micro-organism and the water as fairly safe. If there is only marked fermentation in LA and little or none in either SA, AA and DA, the presumption is that *B. grünthal* is the dominant organism and the water reasonably safe. The critical fermentation is that in DA, and when positive is indicative of the presence of objectionable bacteria, probably *B. oxytocus perniciosus* or *B. coli*, or even both. If the former, then there will be associated fermentation in the three other sugars, while if *B. coli*, then the results will probably show negative fermentation in SA and AA. This latter sequence of results is associated with other micro-organisms than the *B. coli*, and differentiation can only be made by subculturing. But, broadly speaking, this grouping of the fermentations is suggestive of an objectionable type of lactose fractors.

An alternative method to the above would be to omit lactose altogether in the preparation of the stock solutions and substitute

inulin. These, using the corresponding symbols, would be marked SA, AA, DA and IA. Then, following the same routine, we have a series of possible combinations of wider range, and offering a surer basis on which to formulate a definite opinion or interpretation. Assuming we use four series of culture broths containing respectively saccharose, adonit, dulcit and inulin on the plan indicated, then a positive fermentation in all four is a definite suggestion that *B. oxytocus perniciosus* is present. If fermentation is present only in SA, AA and DA, the probable micro-organisms present are Clemesha's types Nos. 66, 67 and 68, the latter being an intermediate in resistance to survival, and the other two unclassified. If fermentation is given only in SA and DA, the probabilities are we are dealing with more or less innocuous types like Nos. 71, 72, 73, 74 and 75. Positive result only in SA suggests *B. coscoroba* and *B. cloacæ*, and possibly presence of unclassified types, such as Nos. 106 and 109. Failure to get fermentation in any of the four series would suggest presence of one or other only of Nos. 4, 5, 6, 7 and 8. Of these No. 4 is *B. grünthal* and No. 6 alone, a doubtfully resistant micro-organism. If fermentation is found only in IA, the presence of *B. levans* and No. 10 is suggested. Given fermentation in SA, AA and IA, we get evidence of sensitive types like Nos. 98 and 99, and a resistant type like No. 97. Similarly, positive results in DA and AA suggest intermediate resistant forms like Nos. 33 and 38. Where DA alone gives a positive result, the presumption favours the presence of objectionable types like *B. coli* and *B. schäfferi*. Positive results only in SA and IA indicate only resistant and presumably harmless varieties of micro-organisms. A heavy pollution by intermediate forms such as *B. lactis aerogenes* and Nos. 100, 101, 102 and 104 are suggested by fermentation only in SA and AA. On the other hand, fermentation only in DA and IA suggests nothing more serious than No. 39, which is unnamed, though of probable recent fæcal origin. *B. acidi lactici* is probably the dominant micro-organism where fermentation is confined only to AA. Given fermentation only in SA, DA and IA, we might suspect Nos. 69 and 70, both unnamed fæcal types but incapable of long survival in water.

Summarizing, the fermentation combinations suggestive of bad or suspicious waters on this rough evidence would be positive results in SA, AA, DA and IA, positive in only SA, AA and IA, positive only in SA, DA and IA, positive only in DA, and IA and a positive only in IA, or a positive only in DA. Of these, the most important and suggestive of definite condemnation are the first and last com-

binations, that is either fermentation in all four of the series or fermentation confined to DA only. The combination of fermentation only in DA and IA is suspicious; the others less so as we do not know yet the degree of danger attaching to such unclassified forms as Nos. 3, 37, 97, 102, 105 and 110 as tabulated by Clemesha. The volume of water from which the various combinations are obtained will naturally influence the interpretation in all cases. In Indian waters, the most important micro-organisms as indicative of recent pollution are *B. oxytocus* and *B. coli* followed closely by *B. schäfferi*. The first named gives a characteristic fermentation combination ; *B. coli* is motile, and *B. schäfferi* is non-motile.

Of course, these are only rough-and-ready procedures and put forward tentatively. For final identification there must be further subculturing, plating and critical examination on well-known lines. These proposed methods are suggested solely as possibly likely to meet the needs of quick work. They are capable of development and improvement. They are advanced here in no spirit of finality or dogmatism, but merely as a possible way out of a difficult situation at this present time, when our knowledge as to the bacterial content of Indian waters is so scanty. Their advancement here must not be interpreted as justifying the abandonment of more elaborate procedures. If we are ever going to acquire complete knowledge of the bacterial contents of Indian or other waters, the examinations must be on orthodox lines and involve subculturing with registration of all the subsidiary reactions. The practical difficulty is the cost of adonit, dulcit and inulin. We are sanguine of overcoming this, as the financial state of all our laboratories in India is likely to improve steadily, and, moreover, we are assured that the Store depots will be able soon to supply these sugars at a reasonable rate, and at prices less prohibitive than those quoted for the small or casual purchaser in the open market.

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ANTELOPE INFECTED WITH *TRYPANOSOMA GAMBIENSE*.[1]

BY CAPTAIN A. D. FRASER, R.A.M.C., AND DR. H. L. DUKE.

THE Sleeping Sickness Commission of the Royal Society, Uganda, 1908-10, showed that waterbuck, bushbuck, and reedbuck could readily be infected with a human strain of *Trypanosoma gambiense*, and that clean laboratory-bred *Glossina palpalis* were capable of transmitting the virus from the infected antelope to susceptible animals.

In the present paper, observations which were made upon these antelope during the eight months subsequent to the Commission's departure from Uganda are recorded. Experiments are also described which show that the duiker—another species of antelope common in most parts of Uganda—can also be similarly infected with a human strain of *T. gambiense*. As regards the antelope employed by the Commission, six of the nine remained in apparently excellent health in April, 1911—roughly, a year after they were infected.

Until bushbuck 2428 escaped from the kraal, and bushbuck 2372 died three hundred and thirty-eight days after its infection as the result of an accident, they had also been healthy. A post-mortem examination was made immediately after death in the case of bushbuck 2372, but no evidence of trypanosomiasis was found.

Reedbuck 2427 appeared to be perfectly healthy for two hundred

[1] Reprinted from the *Proceedings of the Royal Society*. Series (B).

TABLE I.—Giving the Results of Feeding Laboratory-bred *Glossina palpalis* on Antelope infected with *T. gambiense*.

Experiment number	Number of Flies		Number of days after original infection of antelope	Number of days flies fed on antelope	Number of days before flies became infective	Result		Length of experiment, in days	Remarks
	On 1st day	On 30th day				Positive	Negative		
				Bushbuck, Experiment 2328.					
7	?	?	134	5	25	+	..	32	Experiment carried out by Dr. van Someren.
215	115	79	202	10	..	..	–	79	
402	53	18	264	11	..	..	–	64	"Hereditary transmission" flies.
403	49	29	264	11	..	..	–	64	Control to Experiment 402.
538	21	16	306	7	..	..	–	48	"Hereditary transmission" flies.
539	39	31	306	7	..	..	–	62	Control to Experiment 538.
647	52	46	342	6	..	..	–	41	
				Bushbuck, Experiment 2371.					
89	?	?	126	3	50	+	..	59	Experiment carried out by Dr. van Someren.
216	90	64	173	10	..	..	–	78	
404	50	21	235	11	..	..	–	63	"Hereditary transmission" flies.
405	43	31	235	11	38	+	..	51	Control to Experiment 404.
543	33	17	279	9	..	..	–	60	"Hereditary transmission" flies.
544	71	44	279	9	..	..	–	60	Control to Experiment 543.
643	73	49	311	5	..	..	–	42	
				Bushbuck, Experiment 2372.					
88	?	?	123	3	39	+	..	48	Experiment carried out by Dr. van Someren.
218	72	59	171	9	34	+	..	47	
480	79	47	253	7	..	..	–	64	
607	85	61	298	8	25	+	..	33	
658	64	48	315	9	38	+	..	38	
				Bushbuck, Experiment 2428.					
106	?	?	116	2	..	..	–	67	Experiment carried out by Dr. van Someren.
222	20	20	164	9	40	+	..	49	

				Reedbuck, Experiment 2357.					
51	92	18	131	7	..	..	–	77	
189	101	62	173	11	44	+	..	59	
469	83	34	263	9	31	+	..	43	
528	41	20	288	8	..	..	–	57	"Hereditary transmission" flies.
529	70	55	288	8	34	+	..	43	Control to Experiment 528.
631	97	56	322	6	..	..	–	41	
669	119	63	336	12	..	..	–	35	
				Reedbuck, Experiment 2359.					
52	170	64	113	7	38	+	..	55	
190	89	76	155	11	32	+	..	44	
398	50	34	231	12	36	+	..	49	Control to Experiment 400.
400	49	13	231	12	..	..	–	63	"Hereditary transmission" flies.
530	54	19	270	8	..	..	–	61	
642	33	29	306	5	..	..	–	40	
				Reedbuck, Experiment 2427.					
97	60	53	117	8	34	+	..	46	
254	72	52	177	11	29	+	..	49	
				Reedbuck, Experiment 2431.					
98	81	41	119	7	32	+	..	43	
268	60	51	184	10	36	+	.	50	
399	43	29	224	12	..	..	–	63	Control to Experiment 401.
401	25	4	224	12	..	..	–	63	"Hereditary transmission" flies.
598	88	44	288	9	..	..	–	50	
656	66	30	306	11	..	..	–	40	
				Waterbuck, Experiment 2378.					
6	?	?	105	5	25	+	..	32	Experiment carried out by Dr. van Someren.
217	168	53	173	10	..	..	–	68	
406	54	0	237	9	..	..	–	19	
471	96	9	251	7	..	..	–	62	
488	45	28	259	8	..	..	–	59	
523	36	24	270	7	..	..	–	62	
622	62	15	306	7	..	..	–	42	

It will be noted that flies which were fed on bushbuck 2372, 315 days after it had been infected with a human strain of *T. gambiense*, became infected and successfully transmitted the disease to a healthy monkey.

Table II gives the results of the dissections of laboratory-bred *Glossina palpalis* which had fed on the infected antelope.

days after it had been infected. It then died suddenly. At the post-mortem examination performed immediately after death the prescapular glands were found to be the size of a hazel-nut. On section they were hæmorrhagic. There were numerous petechiæ on the mucous membrane of the mesentery. The mucous membrane of the fourth stomach also showed many petechiæ. Microscopical examination of smears made from the various organs was negative. It is, therefore, impossible to say what the cause of death was in the case of this buck.

With the view of ascertaining how long the antelope remained infected, investigations were carried out on the following lines:—

(1) Feeding laboratory-bred *G. palpalis* for several days on the antelope, and subsequently endeavouring to infect a healthy susceptible animal.

(2) Dissecting these flies and examining them for flagellates.

(3) Injecting blood of the buck into animals susceptible to *T. gambiense* infection.

Table I gives the detailed results obtained by the first of these methods. The number of days which elapsed from the date on which the buck was infected until the commencement of the experiment is given.

"Hereditary transmission" flies indicates that the flies before being put upon the antelope had been fed for thirty days upon healthy monkeys to ascertain if laboratory-bred flies which had never fed upon an infected animal could give rise to an infection. As it has been suggested that flies were most readily infected when their first feed was upon an infected animal, these flies were used with the view of obtaining evidence on this point, control experiments being at the same time made with laboratory-bred flies which had not fed before they were put upon the antelope. Although it will be noted that no infection occurred among the "hereditary transmission" flies, whereas the control flies sometimes became infected, the numbers of the flies used are too small to allow of any conclusions being arrived at.

The experiments recorded in Tables I and II are summarized and grouped in Table III according to the length of time the antelope had been infected.

It will be seen that positive experiments were obtained from all the buck (nine examined) when the flies were fed upon them before 200 days had elapsed from the date of the antelope's infection. When more than 200 days had elapsed four of the seven buck examined yielded positive results.

TABLE II.—GIVING THE RESULTS OF THE DISSECTION OF LABORATORY-BRED *Glossina palpalis* WHICH HAD FED ON ANTELOPE INFECTED WITH *T. gambiense*.

Experiment number	Number of flies used in experiment	Number of flies dissected	Number of infected flies found	Percentage of infected flies	Result of experiment	Remarks
			Bushbuck, Experiment 2328.			
7	?	0	0	..	+	Flies not dissected.
215	115	79	0	..	−	
402	53	42	0	..	−	
403	49	42	0	..	−	
538	21	21	0	..	−	
539	39	33	0	..	−	
647	52	51	0	..	−	
			Bushbuck, Experiment 2371.			
89	?	27	0	..	+	
216	90	61	0	..	−	
404	50	33	0	..	−	
405	43	41	0	..	+	
543	33	26	0	..	−	
544	71	66	0	..	−	
643	73	51	0	..	−	
			Bushbuck, Experiment 2372.			
88	?	29	1	3·45	+	
218	72	37	1	2·7	+	
480	79	44	0	..	−	
607	84	70	1	1·4	+	
658	64	59	2	3·4	+	
356	36	36	0	..	−	Experiment lasted 12 days.
357	38	38	0	..	−	,, ,, 14 ,,
			Bushbuck, Experiment 2428.			
106	?	14	0	..	−	
222	20	20	2	10	+	
			Reedbuck, Experiment 2357.			
51	92	30	0	..	−	
189	101	46	4	8·6	+	
469	83	53	5	9·4	+	
528	41	31	0	..	−	
529	70	64	3	4·7	+	
631	97	77	0	..	−	
669	119	65	0	..	−	
			Reedbuck, Experiment 2359.			
52	170	122	0	..	+	
190	89	61	5	8·1	+	
398	50	49	3	6·1	+	
400	49	40	0	..	−	
530	54	23	0	..	−	
642	33	30	0	..	−	
			Reedbuck, Experiment 2427.			
97	60	27	1	3·7	+	
254	72	49	1	2·0	+	
322	28	28	0	..	−	Experiment lasted 9 days.
323	30	30	1	3·3	−	,, ,, 13 ,,
			Reedbuck, Experiment 2431.			
98	81	8	0	..	+	
268	60	53	2	3·7	+	
399	43	36	0	..	−	
401	25	14	0	..	−	
598	88	45	0	..	−	
656	66	35	0	..	−	
			Waterbuck, Experiment 2378.			
6	?	0	0	..	+	
217	168	53	2	3·8	−	
406	54	36	0	..	−	
471	96	18	0	..	−	
488	45	44	0	..	−	
523	36	31	0	..	−	
622	62	20	0	..	−	

Antelope infected with Trypanosoma gambiense

TABLE III.—Summarizing Experiments of Tables I and II and Grouping them according to Length of Time the Antelope had been Infected.

Species of antelope	Days after original infection of antelope	Number of experiments	Number of positive experiments	Number of Flies		Number of flies dissected	Number of infected flies found
				On 1st day	On 30th day		
Bushbuck 2328 ..	100—200	1	1	?	?	0	0
,, 2371 ..	,,	2	1	?	?	88	0
,, 2372 ..	,,	2	2	?	?	66	2
,, 2428 ..	,,	2	1	?	?	34	2
Reedbuck 2357 ..	,,	2	1	193	80	76	4
,, 2359 ..	,,	2	2	259	140	183	5
,, 2427 ..	,,	4	2	190	105	134	3
,, 2431 ..	,,	4	2	209	125	111	2
Waterbuck 2378 ..	,,	2	1	?	?	53	2
Bushbuck 2328 ..	200—300	3	0	217	126	163	0
,, 2371 ..	,,	4	1	197	113	166	0
,, 2372 ..	,,	4	1	238	108	188	1
Reedbuck 2357 ..	,,	3	2	194	118	148	8
,, 2359 ..	,,	3	1	151	66	112	3
,, 2431 ..	,,	2	0	154	74	80	0
Waterbuck 2378 ..	,,	4	0	231	61	129	0
Bushbuck 2328 ..	300—342	3	0	112	93	105	0
,, 2371 ..	,,	1	0	73	49	51	0
,, 2372 ..	,,	1	1	64	48	59	2
Reedbuck 2357 ..	,,	2	0	216	119	142	0
,, 2359 ..	,,	1	0	33	29	30	0
Waterbuck 2378 ..	,,	1	0	62	15	20	0

The results of all experiments are shown in Table IV.

TABLE IV.—Giving Results of Experiments from all Antelopes.

Interval in days after infection of antelope	Number of experiments	Number of positive experiments	Number of flies dissected	Number of flies found infected	Percentage of infected flies
100—200	21	13	745	20	2·7
200—300	23	5	986	12	1·2
300—342	9	1	407	2	0·5
Totals ..	53	19	2138	34	1·5

It appears from the above table that as the interval after the infection of the antelope increases, the percentage of positive transmission experiments and of flies which become infected with flagellates after having fed on the buck diminishes. This diminution becomes still more striking when the results are compared with those recorded by the Commission of experiments carried out soon after the antelope were infected. (Of the twenty-four experiments carried out by the Commission seventeen were positive, 1,722 flies were dissected, and 6·9 per cent. were found to be infected.)

The results of injecting blood of these antelope into susceptible animals are shown in the following table :—

TABLE V.—Giving Results of Injecting the Blood of Antelope Infected with *T. gambiense* into Susceptible Animals.

Antelope	Number of days after infection of antelope	Animal used	Quantity of blood injected in c.c.	Result	Remarks
Reedbuck 2427 ..	200	Monkey	2	Inconclusive	Monkey died. Negative for 9 days.
Waterbuck 2378 ..	306	White rat	1	–	
,, ,, ..	316	Monkey	5	–	
,, ,, ..	316	White rat	1	–	
Bushbuck 2328 ..	355	,,	1½	–	
,, 2371 ..	330	,,	1	–	
,, 2372 ..	327	,,	1	+	Trypanosomes appeared in rat on the 12th day.
Reedbuck 2357 ..	345	,,	1	–	
,, 2359 ..	327	,,	1	–	
,, 2431 ..	320	,,	1	–	
Bushbuck 2372 ..	338	Monkey	5	Inconclusive	Monkey died before trypanosomes could have appeared.

It is seen that the injection of a small quantity of the blood of bushbuck 2372, three hundred and twenty-seven days after it had been infected with *T. gambiense*, produced an infection in a white rat. This, however, was the only positive result which was obtained. Three injections were carried out from waterbuck experiment 2378—on one occasion 5 c.c. of blood was injected—and all were negative. It will be remembered that the Commission found it easy to produce infections in susceptible animals by injecting the blood taken from these antelope soon after they were infected.

Can a Duiker be Infected with a Human Strain of T. gambiense?

Experiment 99, *Duiker.*—On August 30, 1910, 3 c.c. of this buck's blood were injected subcutaneously into a normal monkey to ascertain if the antelope naturally harboured trypanosomes. The monkey's blood was examined regularly for a month. No trypanosomes appeared in its blood, the monkey remaining healthy.

For nine days (January 25 to February 2, 1911, inclusive) laboratory-bred *G. palpalis* known to be infected with a human strain of *T. gambiense* were fed upon the buck.

On February 4, the tenth day after the infected flies first fed upon the antelope, *T. gambiense* appeared in fair numbers in its blood.

Antelope infected with Trypanosoma gambiense

On February 10 and 11, 1911, 119 clean laboratory-bred *G. palpalis* were fed upon the duiker. These flies were subsequently fed on a normal monkey, which they infected after twenty-eight days had elapsed from the date of their first feed on the buck. Of forty-two flies which were dissected, two were found to be infected with flagellates.

Remarks.—The duiker was free from trypanosomes inoculable into a monkey on its arrival at the laboratory.

T. gambiense appeared in the buck's blood on the tenth day after infected flies had fed upon it, and clean laboratory-bred flies successfully transmitted the infection to a healthy susceptible monkey.

Conclusions.

(1) Antelope may remain in apparently perfect health for a year after having been infected with a human strain of *T. gambiense.*

(2) One antelope was still capable of infecting clean laboratory-bred *G. palpalis* 315 days after it had been infected.

(3) A small quantity of blood taken from one antelope 327 days after its infection was proved by inoculation into a white rat to be infective.

(4) As the interval after the infection of the antelope increases, their infectivity as tested by "cycle" transmission experiments, dissection of flies which have fed upon them, and by the injection of the buck's blood into susceptible animals, appears to diminish.

(5) A duiker was infected with a human strain of *T. gambiense* by feeding infected *G. palpalis* upon it.

CELL-INCLUSIONS IN THE BLOOD IN BLACKWATER FEVER.

Second Note.

By Lieutenant-Colonel Sir WILLIAM LEISHMAN, F.R.S.
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Since the publication of my first note on this subject [1] I have had, thanks to the kindness of Sir Almroth Wright and Sir Ronald Ross, the opportunity of studying closely blood-films from two other cases of blackwater fever. It appears desirable to place the result of the examination of this further material on record, on the one hand because it adds somewhat to the significance of the inclusions described in the first case, and, on the other, because it is obvious that, should further experience prove them to have no connection with the disease, the sooner this is established the better, in order that the ground may be cleared for research in other directions.

As the material with which the first note was concerned was derived from a single case, it was naturally encouraging to find in these new cases the same cells and the same inclusions which were described in the first. At the same time, failure to find them would not necessarily have lessened the possible correctness of my suggestions as to their nature, since it is quite conceivable that the bodies found in the cells, whether of Chlamydozoal nature or not, may only rarely be encountered in the peripheral blood, while constantly present in some other situation.

The cases will be briefly described as "Case 2" and "Case 3," that dealt with in the first note being alluded to as "Case 1."

Case 2.—This consisted of a single blood-film, stained by Giemsa's method, which was taken by Dr. Dodgson from a native "boy" suffering from blackwater fever in one of the outlying mines of the Rand, and was most kindly sent to me by my old chief, Sir Almroth Wright. The film showed a very intense degree of leucocytosis; in fact, at first sight, and with a low power, it was suggestive of spleno-medullary leucocythæmia. On closer study, however, the blood picture differed in many respects from that disease. I have no record of the blood count, but a census gave the following relative proportions of the white cells. In making this census there was considerable difficulty at times in assigning a particular mononuclear cell to one of the four heads of "hyalines," "transitionals," "neutrophile myelocytes," and what I classed in connexion with Case 1 as "endothelial cells." Too much stress, then, is not to be

laid upon the strict accuracy of the percentages in connexion with these four groups of cells. There will be noted also a separate heading for what I have called "chrome cells," for the reasons given below.

Blood Census, Case 2.

Polynuclears	63·5 per cent
Lymphocytes	12·5 ,,
Hyalines	2·5 ,,
Eosinophiles	1·5 ,,
Transitionals	5·5 ,,
Myelocytes (neutrophile)	5·0 ,,
Turck's cells	2·0 ,,
Endothelial cells	5·5 ,,
"Chrome" cells	2·0 ,,

In addition to the above, very large numbers of nucleated red cells were present, in the proportion of one megaloblast and four normoblasts to every 100 white cells.

This blood picture makes it evident that the bone marrow in this case was gravely affected, a sign-post which might possibly indicate a useful path of exploration in the future.

No useful purpose would be served by an elaborate analysis of the census, and attention, both in this case and in Case 3, will be confined to certain special points and to the fresh features, or at least features fresh to myself, disclosed in each instance.

The cell-inclusions, described and sketched in connexion with Case 1 were found to be plentiful in this film. The cells in which they were encountered appeared to be of the same nature as those which were classed as endothelial cells in Case 1, and none were encountered in any of the ordinary leucocytes found in normal blood. The type of inclusion most commonly met with was that figured in Nos. 1 to 4 of the coloured plate, to which reference may be made. They stained a varying depth of pink or red, and were almost invariably clear cut and circular in contour. The forms were mostly homogeneous, the ring forms which were fairly common in Case 1 being rare in Case 2. None of the large forms, as shown in fig. 13 of the coloured plate, were found in this film.

In connexion with the various possibilities as to their origin, analysed in the former note, it was stated that the greatest difficulty was found in deciding whether they might not be altered or fragmented red cells which had undergone phagocytosis: this difficulty was even more apparent here, since undoubted phagocyted reds were found in some of these large mononucleated cells and a few even in cells which contained inclusions. In spite of this fact I

still consider that the inclusions are not explicable on this ground, and the more I have seen and studied them the more do I feel convinced of this.

The granules mentioned in the former note, as occurring in many of the cells which showed inclusions, were also found in this instance, though by no means invariably. Comparison between the granules of the two cases was rendered difficult by reason of the different staining methods employed. In Case 2, however, a remarkable feature of many of the mononucleated cells was the presence, often in great numbers, of granules in the cytoplasm which I was unable to refer to any of the types of Ehrlich or to the azure granules of normal lymphocytes or hyalines. These granules were deep red, and displayed an intense affinity for the chromatin element of the dye; they were a little larger than neutrophile granules, though not nearly so large as coarse eosinophile or basophile granules. Their distribution in the cell cytoplasm was patchy; while some cells were almost filled with them, others would show only a small clump localized in one portion of the cytoplasm. These granules were encountered in cells of several different types, but were never seen in polynuclears or in eosinophiles. It is possible tbat they represent a stage in the history of a Chlamydozoon, but this remains at present purely conjectural.

In both this case and Case 3 certain curious cells were found which I have never previously encountered, either in blood-films or in plates illustrating cytological work. These cells were not uncommon and could be readily distinguished from all others, even with a low power, by the deep chromatin tint of the whole cell; they were of the size of ordinary polynuclears and their nuclei were sometimes of that type, sometimes mononuclear. The cytoplasm appeared more or less completely filled with material which showed the chromatin reaction and, in almost every instance, the red colour was most intense at the periphery, giving the cell an appearance of being capsulated. In other instances the partial disruption of the cell permitted it to be seen that the red-staining material consisted of a mass of chromatin bodies, sometimes of quite irregular shape and size, but occasionally showing a tendency to ring form. It is difficult to convey the appearance of these cells apart from a coloured sketch, but the accompanying photographs (see figs. 1 and 2) give a fairly good idea of their general appearance. In each instance a cell was selected which was close to an ordinary polynuclear that the latter might serve as an index of comparison. For the sake of avoiding frequent periphrases I may perhaps be pardoned labelling

them provisionally "chrome cells." They will be mentioned again in connexion with Case 3.

Case 3.—The two stained films from this case were lent to me by Sir Ronald Ross, to whom I am further indebted for his interest in and criticism of my former note. These films have the further interest of being derived from the interesting case of which he published particulars, with Drs. D. Thomson and G. C. E. Simpson, at the end of 1910 [2]. In view of the full details there given, I need only mention that this case occurred under their own observation at Liverpool, and led them to the conclusion that neither the hæmoglobinuria nor the subsequent attacks of fever from which the patient suffered could be attributed to the toxins of malarial parasites.

Each of the films had been stained by Giemsa's method. As recorded in the chart of the case, 3,000 white cells per cubic millimetre were found on the day on which the films were taken, which was at the commencement of the third attack of fever, during which there was no hæmoglobinuria and no malarial parasites were found. For the sake of uniformity I may give the result of my own census of the white cells :—

Blood Census (*Case* 3).

Polynuclears	63·0 per cent
Lymphocytes	13·5 ,,
Hyalines	7·0 ,,
Eosinophiles	1·5 ,,
Transitionals	7·0 ,,
Myelocytes (neutrophile)	3·0 ,,
Endothelial cells	4·5 ,,
"Chrome" cells	0·5 ,,

In this case, possibly owing to the leucopenia which existed, and the smaller number of endothelial cells, inclusions of the type found in Cases 1 and 2 were rare, only two or three cells showing them; those found were of the small homogeneous type figured in Nos. 1—4 of the coloured plate.

The special interest, however, of these two films was: 1st, that each showed a fair number of the "chrome cells" described in connexion with Case 2 and precisely identical with those in appearance, size and staining reaction; and, 2nd, that, in the case of one of the films, somewhat more deeply stained than the other, two cells were encountered which appear to suggest a possible connexion between the inclusions, so frequently alluded to, and the "chrome cells." Each of these cells I have photographed (see figs. 3 and 4), and the reproduction will show that, in each instance,

FIG. 1.

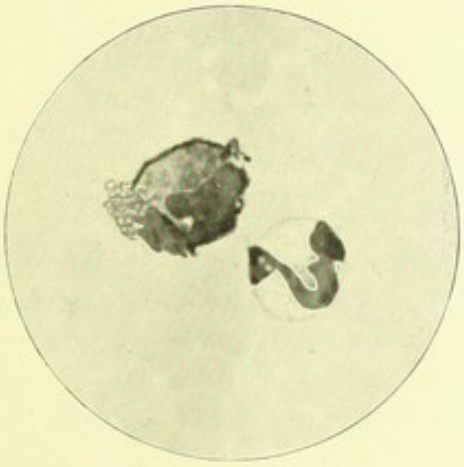

FIG. 2.

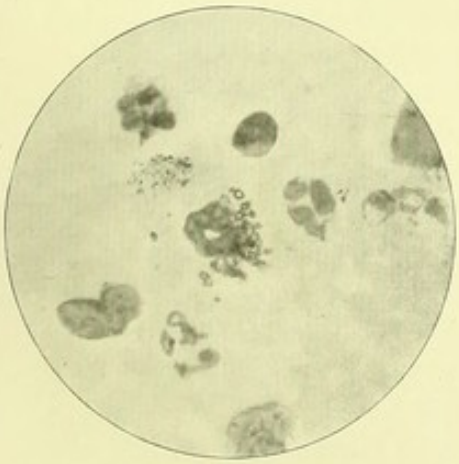

FIG. 3.

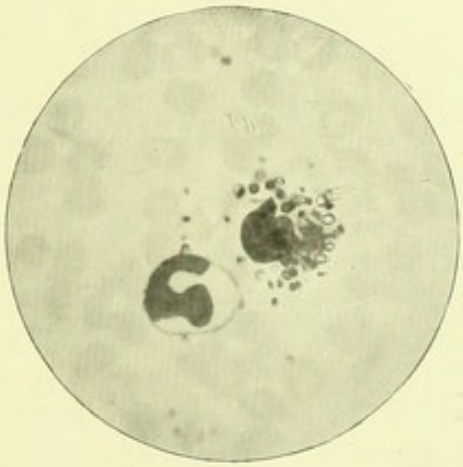

FIG. 4.

To illustrate "Cell-inclusions in the Blood in Blackwater Fever."

By Lieut.-Col. Sir WILLIAM LEISHMAN, F.R.S., R.A.M.C.

the cell wall has been ruptured and has liberated a number of deep-staining chromatin bodies, of varying size and showing a pronounced tendency to ring form. Contrasting these with the photograph of the "chrome cell" in fig. 3, where some of the chromatin contents of the cell are sufficiently isolated to show a tendency to similar ring form, it is hard to resist the impression that the well-marked bodies seen in the disrupted cells in figs. 3 and 4 represent a further stage in development of the elements which compose the red staining mass which fills the protoplasm of the "chrome cells" found in Cases 2 and 3. Again, as far as one can judge by staining reaction, size and shape, criteria of admitted insufficience, these chromatin rings are the same as the ring forms described in the endothelial cells in Case 1. I may add, since I have used the term "ring form," that the bodies in question show no resemblance to the ring forms of malarial parasites and could not possibly be confused with the latter.

As to the possible occurrence of similar inclusions in other conditions, Dr. G. C. Low [3] has recently recorded that he has seen bodies, apparently similar to those which I described, first in some cases of fever from Borneo, and second, in the blood of pellagra cases recently brought home by Dr. Sambon from Italy. I have not seen the specimens alluded to in Dr. Low's article, but I had recently an opportunity of discussing the subject with him, and of showing him a few of the inclusions from Case 1, and I think it possible that the smaller granules which he noted and has figured in mononuclear cells may have been azure granules. In these three cases of blackwater, azure granules were common in both lymphocytes and hyalines, but were quite distinct from the inclusions. It is quite possible that the larger inclusions of which Dr. Low speaks may resemble those in question here, but without seeing them I can express no opinion.

Major W. S. Harrison has, however, shown me a blood-film from a case of chronic malaria, in which there were present in large cells, of mononuclear type, homogeneous, pink-staining inclusions, which I agree with him in regarding as identical with the blackwater ones. In this case he said there was no history of blackwater and no probability of its development, but it appears to me of some significance that the officer in question had recently returned from Nigeria, where he contracted his malaria, and that blackwater fever is common in that country. Making the large assumption that the inclusions may eventually prove to be, or to be due to, the specific cause of this disease, it is quite probable that

it is only under certain, as yet unknown, conditions that they give rise to the symptom of hæmoglobinuria.

Should the further material which I hope to receive before long disclose any facts of fresh interest, either favourable or inimical to my views, they will form the subject of another note; the present one, as may be seen, is no more than a progress report, and any attempt to expand the hypothesis put forward in my former article appears unjustifiable in view of the paucity of the material.

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FURTHER EXPERIMENTAL INVESTIGATION INTO SUDAN KALA-AZAR.

By Captain W. E. MARSHALL.
Royal Army Medical Corps.

In previous communications on Sudan kala-azar it has been shown that this form of Leishmaniasis has the following characteristics:—

(1) It affects people of all ages, but is much commoner in late childhood and early adult life.

(2) The *Cercopithecus sabæus*, the ordinary grey monkey of the Sudan, can be infected with the disease by intraperitoneal or subcutaneous injection of the parasite.

(3) The parasite grows readily on 10 per cent citrate, on Novy and MacNeal's medium and on Nicolle's medium.

(4) The parasite can be demonstrated in the peripheral blood of the infected in a large percentage of the cases.

In the study of the method of transmission of infantile Leishmaniasis considerable progress has been made. The similar geographical distribution of canine and human Leishmaniasis and the occurrence of human cases in close contact with canine cases made it extremely probable that infection was conveyed from dog to child. There is now considerable experimental evidence in support of this theory, the probable transmitter being the *Ctenocephalus canis*, the dog-flea. Basile was successful in transmitting the disease from infected to uninfected pups, and was also able to infect pups with fleas brought from an infected district. The parasite has also been demonstrated in the interior of fleas by Basile, Sangiorgi, and Alvarez and Da Silva. Basile, La Cava and Visentini have found them also in *Pulex irritans*, the human flea. Franchini and Gabbi, on the other hand, have failed to find any intracorporeal development in the body of the flea. In support of the dog-flea theory it may be mentioned that the Sergents, Lombard, and Quilichini have recently found an infected kitten living in the house containing an infected child and an infected dog.

Present Investigation.

The following experiments are a continuation of the work already published with regard to the clinical features of the disease. There is little to add, but it is necessary to modify the statement

that the parasite is always present in the peripheral blood in small numbers, as two cases were seen where the parasites were present in considerable numbers. In one case, shown in the plate, there were two mononuclear cells in one slide, one containing thirty-two parasites and the other twenty-nine. This was early in the course of the disease. In the other case the parasites were extremely numerous, but were more uniformly distributed among the white blood-cells, the majority being inside polynuclear cells. This was from a case in the advanced stages of the disease.

The diminution or absence of eosinophile cells from the peripheral blood is usually well marked, and is of some help in differentiating kala-azar from malarial infections where usually a slight eosinophilia exists.

No drug has been found which in any way affects the course of the disease, and all cases seen since our last report have terminated fatally; "606" was tried in one case, but without any benefit.

Experimental Kala-azar in the Dog.

Our previous inoculation experiments with dogs were inconclusive, as with one exception all the dogs died soon after inoculation. We have therefore again inoculated four dogs with the parasites and in each case a positive infection was obtained.

Dog 1, ♂, injected intraperitoneally with post-mortem spleen emulsion from infected monkey.

82nd day: Liver puncture positive.

145th day: Chloroformed, infected with Leishmania.

Dog 2, ♂, pup, injected intraperitoneally; material obtained by post-mortem spleen puncture from a human case.

Died on 128th day; heavily infected with Leishmania.

Dog 3, ♂, pup, injected intraperitoneally with post-mortem spleen emulsion from Dog 1.

Died on 95th day; infected with Leishmania.

Dog 4, ♂, pup, injected intraperitoneally with material obtained by spleen puncture from a human case.

Died on 74th day; heavily infected with Leishmania.

There is therefore no doubt that dogs are susceptible to Sudan kala-azar. They can be infected from the monkey, from another infected dog, or from human cases. Young dogs are more susceptible, and in them the disease runs a more acute course. Dog 1, which was an adult, showed very few symptoms, and was in fairly good condition on the 145th day when it was chloroformed. As in the monkey, probably the best way of producing an infection is from

a spleen puncture of a human case during life. Dog 4 was inoculated in this way, and died with a severe infection on the 74th day.

Owing to Basile's discoveries it was decided to try to convey the disease from dog to dog by means of fleas, the *Ctenocephalus canis* being used.

Dog 5, ♂, was put in the room beside Dog 1 after the latter was known to be infected. The dogs were in contact for thirty-seven days, and fleas were frequently placed in the room and were constantly present on both dogs. No infection occurred. This dog was chloroformed five months later, when no parasites were found on post-mortem examination.

Dog 6, ♀, pup, was put in the box beside Dog 2, four days after the latter had been inoculated. They were together for 124 days and fleas were frequently placed in the box. No infection occurred. This dog was chloroformed two months later; the post-mortem examination showed filaria, but no Leishman-Donovan parasites.

So far, therefore, we have been unable to convey infection from dog to dog by means of the flea. It must be borne in mind, however, that these experiments were carried out in the summer, when human cases are less frequent, and when the temperature is probably less favourable for infection.

A similar experiment was carried out to determine if ticks could convey the disease from dog to dog.

Dog 7, ♂, pup, was put in box beside Dog 3, when the latter was inoculated. The dogs were 95 days in contact. Ticks were introduced at frequent intervals. No infection occurred in Dog 7.

Experimental Kala-azar in the Grey Monkey (*Cercopithecus sabæus*).

Further experiments were carried out to endeavour to determine by what means the disease may be conveyed from monkey to monkey.

In one experiment an infected and a healthy monkey were freed from all insects and were kept together in a wire-gauze cage. They were fifty-four days in contact. No infection occurred in the healthy monkey.

In a second experiment similar precautions were taken, but monkey fleas were placed in the cage. Unfortunately the infected monkey died twelve days later, so they were only a short time in contact. No infection occurred in the healthy monkey.

In a third experiment an infected and a healthy monkey were

freed from all insects and kept in an open cage. They were seventy-one days in contact. No infection occurred in the healthy monkey.

In a fourth experiment no precautions were taken to render the monkeys free from insects, and one infected and two healthy monkeys were put together in an open cage. There was a doubtful infection of one healthy monkey, but the slides were unsatisfactory, the post-mortem examination being made some time after death.

In a fifth experiment, similar to the fourth, no infection occurred in the healthy monkeys.

In a sixth experiment numerous lice (*Pediculus capitis*) were fed at intervals on a human case and then transferred to a healthy monkey. No infection occurred.

These results are therefore all negative, with the possible exception of one monkey in Experiment 4. In that experiment lice were the only insects actually found. [The monkey louse (*Pedicinus*) is quite different from the human species, having a very elongated head and only three instead of five joints to the antennæ.] In this experiment also the infected monkey was removed from the cage for a time in order to see if the *Ctenocephalus canis*, the dog-flea, would infect the monkey, though with apparently negative results. As the cage was an open one mosquitoes also were not excluded, so that even if this monkey were infected, the louse, the flea, and the mosquito are all possible carriers of infection.

In the autumn many of the monkeys suffered from the plasmodium of monkey malaria, and we lost many animals.

Though one can, in the majority of cases, be sure of infecting monkeys, we had one animal which showed a natural immunity from infection. It was injected intraperitoneally from spleen puncture of a human case, but no infection occurred. Six months later it was again inoculated intraperitoneally with a spleen emulsion from Dog 1, and again no infection took place. Another monkey inoculated with the same splenic emulsion contracted the disease. Perhaps, as Delanoë has recently shown occurs with cultures of Leishmania, the immunity is purely phagocytic.

Conclusions.

(1) Dogs can be experimentally infected with Sudan kala-azar.

(2) They can be infected from human cases, from infected monkeys, or from other infected dogs.

(3) Young dogs are more susceptible, and in them the disease runs a more acute course.

(4) Experiments to transmit the disease from dog to dog by means of the *Ctenocephalus canis*, the dog-flea, gave negative results.

(5) The Leishman-Donovan parasite is occasionally present in large numbers in the peripheral blood of human cases.

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Drawings.—Fig. 1. Large mononuclear cell from peripheral blood, case of kala-azar; it contains thirty-two parasites. × 1,500. Giemsa's stain.

Fig. 2. Cell from same slide, containing 29 parasites. × 1,500. Giemsa's stain.

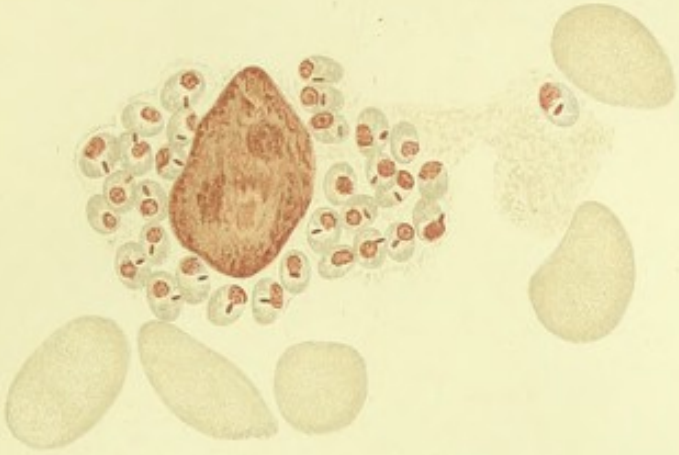

Fig. 1. Peripheral Blood. × 1,500 × × ×

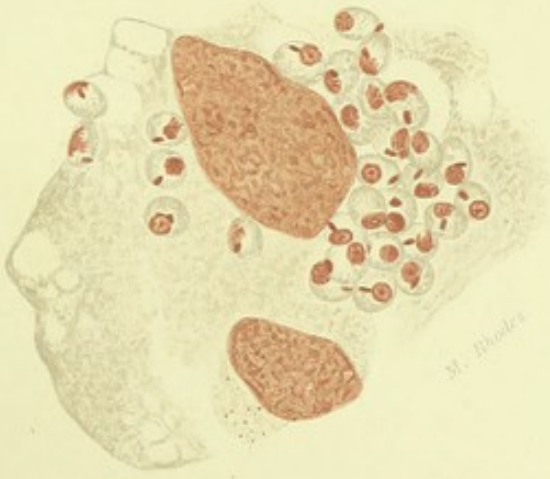

Fig. 2. Peripheral Blood. × 1,500 × × ×

To illustrate "Further Experimental Investigation into Sudan Kala-Azar."
By Captain W. E. Marshall, R.A.M.C.

VOLUME XIX. SEPTEMBER, 1912. No. 3.

Journal

of the

Royal Army Medical Corps.

Original Communications.

NOTE ON A BACILLUS OCCURRING IN SOME INTRACTABLE VENEREAL ULCERS.

BY LIEUTENANT C. H. H. HAROLD.
Royal Army Medical Corps.

AMONGST ulcerative venereal diseases not the least important is the condition known as soft chancre, a term applied to numbers of non-syphilitic lesions which are usually attributed to infection with Ducrey's bacillus. Clinically these cases present great differences, and it is questionable whether in some of them at any rate another organism is not the chief factor in their causation. Cases of this type of disease may be divided into two main groups.

Group (1)—Soft ulcers which remain in a callous condition for a few weeks and may give rise to a bubo requiring operative treatment. Surgical measures as a rule are quite sufficient to cause healing of these cases in a comparatively short time.

Group (2)—A more eroding type of ulceration, with which this paper is more intimately connected; this form is much more resistant to treatment, spreading in spite of every conceivable surgical procedure, and may destroy considerable portions of the external genitalia. The base of the ulcer is usually soft and covered with greyish granulations which secrete a viscid glutinous pus with a characteristic odour, while its cleanly cut and slightly undermined edges show no signs of reaction. These ulcers frequently cause inflammatory buboes which require

operative interference and the resulting wound exhibits the same ulcerative characteristics as those of the primary ulcer. Some buboes also which depend upon soft chancres of a simpler type than the one just described may take on this character after being opened.

This second type of ulcer is important because of its unsatisfactory response to treatment. The patient remains in hospital for many weeks or even months, being not only inefficient but causing the expenditure of considerable time and energy on the part of his medical attendants.

On account of the unsatisfactory results of purely surgical treatment it was decided to investigate the bacteriological nature of these lesions with a view to vaccine treatment. The following cases in which this was done will illustrate the value of this procedure.

A patient under the care of Major C. W. Profeit, R.A.M.C., was suffering from a slow phagedænic ulceration of the penis clinically resembling the second group of cases just described. Two-thirds of the glans penis had ulcerated away and the remaining third was attached by a small ulcerated pedicle. The ulcer also extended the whole way around the body of the penis and following the course of the urethra had penetrated for some distance into the corpus. The passage of urine irritated the ulcer and gave rise to much pain and scalding. He was also suffering from syphilis contracted at the same time. In another hospital, treatment which included three operations and vigorous anti-syphilitic measures had been carried out for seven months, but had been unsuccessful in arresting the destruction of tissue. After transfer to Rochester Row, he had been treated with salvarsan and mercury and clinically showed no signs of syphilis, while his blood for the last three months had given a negative Wassermann reaction. In spite of all this medical and surgical treatment, however, the ulceration was becoming progressively worse, and as a last resource amputation of the penis was advised; to this the patient cheerfully agreed :—

Before doing this Major Profeit suggested that it might be of interest to make a complete bacteriological investigation of the ulcer with a view to vaccine treatment. The edge of the ulcer was scraped and some of the exuded serum examined by the dark ground illumination, but no spirochætes of any description were discoverable. Some of the exuded serum was sown on several tubes of human serum agar and two kinds of organisms were

recovered from them: (1) a *Staphylococcus aureus;* (2) a short bacillus. A carbolized vaccine prepared from the bacillus was injected into the patient with rather astonishing results.

On the same evening the penis became so swollen and painful that the man was unable to sleep, and on the following day the base and edges of the ulcer had assumed a bright red colour, contrasting with their previous pale appearance, while the dressing showed an increased exudation of pus. There was also a large red tender swelling at the site of inoculation.

From this day onwards the ulceration improved and commenced to heal. The vaccine was repeated after four days and was followed by a reaction of the same type, but not so severe as after the first injection. Repeated doses of vaccine were subsequently given at four-day intervals and within a month the whole of the ulceration had healed with the exception of a small ulcer on the glans penis. The healing of this was probably delayed because the scar tissue at the base of the glans interfered with its blood supply. The patient refused to have this small portion removed, having made such rapid progress under vaccine treatment, and the small ulcer, a few millimetres broad, delayed his discharge from hospital for another month.

A few days after commencing the treatment of this case with vaccine the ulcer was again scraped and tubes inoculated with exuded serum. The same bacillus was recovered, but this time in pure culture.

My next patient was suffering from a chronic ulcerating bubo; he had been in hospital for sixteen weeks and showed no signs of improvement. Following the same method of procedure as in the previous case, the ulcer was scraped and the same organism recovered associated with a diplococcus. The same vaccine was administered and a marked reaction followed. On the night of the injection the man complained of severe pain in his thigh and was unable to sleep. The next day the whole of the groin was swollen and the bubo of a bright red colour, discharging increased quantities of pus; a large localized, red, tender swelling had also formed at the site of inoculation. Similar reactive signs, but of less severe character, occurred after each successive injection of vaccine; the man was discharged cured in less than a month from the time of commencing the vaccine treatment.

The next case clinically resembled the previous one. The patient had been in hospital for six weeks and the ulcerating bubo remained in a callous condition. The same bacillus and the

S. aureus were isolated and injections of vaccine caused the usual reactive signs in the patient, who was discharged cured in five weeks from the time of commencing the treatment.

The last patient was also suffering from an ulcerating bubo which had kept him in hospital for four weeks and bacteriological examination revealed the same organism associated with a *S. albus*. He gave the usual response to vaccine and the bubo is now rapidly healing up. Owing to my unavoidable absence from hospital the patient received one dose of vaccine instead of three in sixteen days, and during that time the bubo showed no signs of improvement.

These cases have all been treated by a vaccine made from the bacillus isolated from the first patient and grown on agar. The vaccine was sterilized by the addition of 0·5 per cent carbolic acid. The initial dose was 15 millions and this was increased gradually to 2,000 millions, the intervals between injections being four days. These have all caused marked local reactions both in the site of infection and in the site of inoculation. They were followed by little or no constitutional disturbance, the temperature of the patient rarely rising more than a degree. When for some reason the injections were withheld, or were not strong enough, the lesions lapsed to the previous callous condition. On these occasions the administration of stronger or more frequent doses of vaccine always produced a beneficial effect.

In none of the cases from which I have recovered this bacillus have I been able to find any organism corresponding to Ducrey's bacillus. The local reaction at the site of the ulcer and the marked benefit which was obvious from the commencement of vaccine treatment are strong evidence that the bacillus in question was the chief ætiological factor in the production of the disease.

Morphologically, it is a short rod-shaped organism 2·5 to 3 μ long and about 0·3 μ broad. In film preparations it is frequently seen lying in parallel rows, giving one the impression of a short palisade. It stains readily with any of the basic aniline dyes and is Gram positive. With all stains, but especially with carbol thionin, it shows marked bi-polar staining with a clear interval between the granules. These can also be demonstrated in many individual bacilli with Neisser's stain. It does not form chains, nor spores. Involution forms are frequent in cultures a few days old, the most frequent variety of these being swollen elongated forms which show no polar staining. Another form frequently seen is somewhat clubbed at one end, which is occupied by a large polar granule.

The organism is strictly aerobic, growing very luxuriantly on human serum agar as well as on 2 per cent agar (+ 6 Eyre's scale). After twelve hours incubation at 37° C., the colonies are the size of a pin's head, moist and heaped up in the centre. By reflected light they appear greyish white and with transmitted light they show a yellow centre with a lighter, well-defined margin. On microscopical examination with the low power they are finely granular with a sharply defined evenly circular margin. The colonies tend to remain discrete; in four days they attain a diameter of 3 mm. and their centres become a deeper yellow colour.

Grown on human serum it forms a grey white deposit and flocculi which sink to the bottom.

It does not liquefy *gelatine;* it grows on neutral red agar, but causes no change in the medium.

In peptone salt solution no indol is formed, while litmus milk is bleached by its action.

It ferments glucose and cane sugar, forming acid but no gas.

In lactose, maltose, dulcite, raffinose, inulin, and salicin no change is produced.

It is non-pathogenic to rabbits when injected into the blood-stream and only causes a local infiltrate when injected beneath the skin of the animal's ear.

In the "Bacteriology of Diphtheria," by Nuttall and Graham Smith, G. S. Graham Smith has collected the observations of numerous workers on the recovery of diphtheroids from the male and female genital tract. The chief varieties with their essential points of difference or similarity are as follows:—

Neisser (1888) recovered from the female vagina diphtheroids which were motile.

Berghy (1898) isolated from the urine and vaginal discharges of certain cases a Gram-positive diphtheroid which differed from the present one by forming a yellow growth on agar, by not liquefying gelatine and by forming a white membrane on broth. It was non-pathogenic to guinea-pigs and did not form acid in glucose.

Foulerton and Bonney (1903) recovered from two cases of puerperal septicæmia and from a case of phagedæna of the penis a Gram positive diphtheroid which showed no granules when stained with Neisser. Stroke cultures had the appearance of ground glass, and when grown in broth it appeared as an evenly staining rod. It did not ferment glucose and was non-pathogenic to guinea-pigs.

Pfeiffer in 1903 isolated Gram negative diphtheroids from normal men and from cases of gonorrhœa.

Graham Smith, Hallé, and Robertson and McRae have also recovered diphtheroids of a more diverse character.

On referring to the original literature I cannot find evidence that any test to prove the pathogenicity of these organisms to man was carried out, or that vaccines prepared from them exercised any therapeutic effect.

In conclusion, I wish to thank Major C. W. Profeit, R.A.M.C., for bringing these cases to my notice, and Major L. W. Harrison, R.A.M.C., for conducting the animal inoculations, and for his valuable technical suggestions.

NOTE.—Since writing the above my attention has been drawn to an article by Herbst and Gatewood, of Chicago, in the *Journal of the American Medical Association* for January 20, 1912. These workers made a bacteriological examination of a series of twenty-six soft sores, and recovered diphtheroid bacilli from sixteen of them, while a doubtful Ducrey's bacillus was found on two occasions only. A vaccine prepared from the supposed Ducrey's bacillus was administered to several patients, but with very poor results. One of the patients became steadily worse and was eventually treated with vaccine prepared from a diphtheroid bacillus and a staphylococcus isolated from the lesion. The result was excellent and subsequently other cases were treated with the same vaccine whenever an autogenous vaccine could not be prepared.

This vaccine gave rise to reactions which were exactly similar to those which I have described in my cases, but the authors do not state quite clearly in how many of the cases beneficial results followed its administration. Altogether they treated thirty-eight cases, of which thirty-nine per cent were benefited by vaccine treatment, but some of the thirty-eight were treated only with the vaccine prepared from the supposed Ducrey's bacillus. They believe, however, that if the diphtheroid or an autogenous vaccine had been used throughout the series the results would have been much better.

Guinea-pigs inoculated with a diphtheroid bacillus isolated from one of the cases developed local infiltrates; on one occasion the axillary glands became enlarged, and on another an ulcerating lesion formed at the site of inoculation from which the same organism was recovered.

They conclude that the diphtheroid bacilli isolated were the

cause of the soft sores in these cases, although organisms which are morphologically identical are commonly present in the urinary tract. They have not, however, detailed the morphological and cultural characteristics of the several organisms they isolated, so that it is impossible to identify any of them with the bacillus isolated at Rochester Row.

It seems probable, however, that the diphtheroid organism from which these workers prepared their vaccine is identical with the one which I isolated. Their methods of isolation, which were similar to mine, demonstrated the persistent presence of a diphtheroid, and the response to vaccine prepared from this micro-organism was the same as in my cases.

Their observations fully confirm those which I have independently made in indicating that soft chancres are not always caused by Ducrey's bacillus, and that the additional work expended in making a bacteriological examination of these cases with a view to vaccine treatment is amply repaid.

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SALVARSAN AND NEOSALVARSAN IN THE TREATMENT OF SYPHILIS.

By Brevet-Lieutenant-Colonel T. W. GIBBARD, Major L. W. HARRISON and Lieutenant A. S. CANE.
Royal Army Medical Corps.

The importance of syphilis to the Army justifies the following further report on our observations regarding its treatment with salvarsan.

The following subjects are discussed in this paper: (*a*) The value of salvarsan as compared with that of an exclusively mercurial therapy in reducing the inefficiency of soldiers which is caused by syphilis; (*b*) the value of using mercurial injections in conjunction with salvarsan as compared with that of relying entirely on salvarsan; (*c*) the importance of early diagnosis and treatment; (*d*) the dosage of salvarsan; and (*e*) neosalvarsan. Some cases of re-infection which have occurred after salvarsan treatment will also be described.

In a previous paper we have shown that syphilitic soldiers who are treated with salvarsan, either alone or in conjunction with a course of nine mercurial injections, suffer considerably less from relapses and spend a shorter time in hospital during the first nine months of their disease than those who are treated exclusively with mercury.

We have now watched our salvarsan cases for considerably longer periods, and the results of our extended observations, which are shown in the following tables, prove the immense advantage of a salvarsan therapy over an exclusively mercurial one.

To avoid any possible bias in favour of salvarsan, the figures in these tables which relate to mercurial treatment have been taken from all the case sheets we could find of men who were treated with not less than nine injections of mercurial cream in the first course, rested for not more than eight weeks, received six injections in the second course, and were, in fact, thoroughly treated with mercury throughout the period of observation.

Table I shows the relapses which occurred amongst syphilis cases which were treated from the outset with salvarsan and the periods during which the patients were under observation.

We would draw attention to the marked difference which is illustrated in this table between the numbers of relapses which

occurred under mercurial injections on the one hand, and those which followed a combined course of two intravenous injections of salvarsan and nine intramuscular of mercurial cream, on the other. (See six to twelve months' period.)

TABLE I.—Showing the Number of Fresh Cases of Syphilis treated with (*a*) Salvarsan, either Alone or in Conjunction with Mercurial Injections and (*b*) Mercurial Injections Only and the Number of Relapses which occurred at Different Periods under each Form of Treatment.

		All cases				Primary cases only			
Period under observation	Method of treatment	Total cases	Total clinical relapses	Required re-admission to hospital	Treated with more salvarsan subsequently to the first course because of positive Wassermann without clinical relapse	Total cases	Total clinical relapses	Required re-admission to hospital	Treated with more salvarsan subsequently to the first course because of positive Wassermann without clinical re apse
6—12 months	Intramuscular of salvarsan	10	3	2	4	5	1	1	1
	1—4 intravenous of salvarsan	74	8	1	7	24	2	1	0
	2 intravenous of salvarsan and 9 intramuscular of mercury	104	3	0	2	40	1	0	0
	Mercurial injections only	102	85	–	–	38	36	–	–
12 to 23 months	Intramuscular of salvarsan	9*	2	1	6	4*	1	1	1
	1—4 intravenous of salvarsan	48*	9	1	4	17*	1	1	0
	2 intravenous of salvarsan and 9 intramuscular of mercury	20*	1	0	1	7*	0	0	0

* These are included in the corresponding totals under the 6 to 12 months' period.

Table II shows the number of primary cases treated with salvarsan and with mercury respectively, who developed secondary symptoms within periods of observation which ranged from six to twenty-three months. The thirty-six patients referred to in this table who were treated entirely with mercurial injections and who developed secondary symptoms did so in an average period of seven weeks so that it is fair to mention also that out of 101 patients whom we have treated with intravenous injections of salvarsan, and have observed for three months or longer, two only have

developed secondaries. In each of these primary cases the diagnosis was established by microscopical examination of the exudate from the sore.

TABLE II.—Showing the Number of Primary Cases which Subsequently developed Secondary Symptoms under each Form of Treatment: (*a*) Salvarsan, either Alone or in Conjunction with Injections of Mercurial Cream, and (*b*) Mercurial Injections Only.

Period under observation	Method of treatment	Total cases	Total which developed secondaries
6—12 months	Intramuscular of salvarsan..	5	1
	1—4 intravenous of salvarsan	24	1
	2 intravenous of salvarsan and 9 intramuscular of mercury	40	1
	Mercurial injections only ..	38	36
12—23 months	Intramuscular of salvarsan..	4*	1*
	1—4 intravenous of salvarsan	17*	1*
	2 intravenous of salvarsan and 9 intramuscular of mercury	7*	0

* These are included in the corresponding numbers under the 6 to 12 months' period.

Briefly, the tables show that in periods ranging from six to twelve months the clinical relapses under exclusively mercurial treatment were 11·5 times as many as under salvarsan, while the proportion of primary cases which subsequently developed secondaries under exclusively mercurial treatment was thirty times as great as under salvarsan.

The Wassermann reactions given by patients who were treated from the outset with salvarsan are shown in Table III and those given by patients at different stages of the regular mercurial treatment are shown in Table IV.

In comparing the results in Table III with those in Table IV, it must be remembered that, other things being equal, a higher percentage of positive reactions is to be expected in the first than in subsequent years of the disease, and that the number of positive reactions tends to rise the longer the interval which has elapsed since treatment was suspended. The cases shown in Table III (salvarsan) were for the most part in the first year, and the intervals which had elapsed since treatment was suspended were greater than any shown in Table IV. Yet the percentages of positive reactions were lower in the salvarsan series than at any period shown in the mercurial series. With regard to the latter, the proportion of cases which gave a positive reaction three months after the termination of two years' regular treatment is significant.

TABLE III.—Wassermann Reactions, by the Original Method, given by Fresh Cases of Syphilis at Various Intervals of Time after suspending Treatment with Salvarsan.

Clinical stage of disease on commencement of treatment	Method of treatment	4—7 months				7—10 months				10—18 months			
		Total cases	Positive	Negative	Per cent positive	Total cases	Positive	Negative	Per cent positive	Total cases	Positive	Negative	Per cent positive
Primary and secondary	1 subcutaneous or intramuscular	9	2	7	–	4[1]	2	2	—	4[2]	0	4	–
	1—4 intravenous	72	17	55	23·6	37[3]	9	28	24·3	21[4]	4	17	–
	2 intravenous of salvarsan and 9 intramuscular of mercury	76	6	70	7·9	21[5]	2	19	9·5	4	1	3	–
	Totals excluding subcutaneous and intra-muscular	148	23	125	15·5	58	11	47	18·9	25	5	20	20
Primary only	1 subcutaneous or intra-muscular	5	1	4	–	2	2	0	–	2	0	2	–
	1—4 intravenous	21	3	18	14·2	9	1	8	–	7	1	6	–
	2 intravenous of salvarsan and 9 intramuscular of mercury	32	0	32	0	6	1	5	–	2	0	2	–
	Totals excluding subcutaneous and intra-muscular	53	3	50	5·6	15	2	13	–	9	1	8	–

[1] Excluding two cases under observation for this period, but treated with more salvarsan.

[2] Excluding one case under observation for this period, but treated with more salvarsan.

[3] Excluding one case under observation for this period, but treated with more salvarsan for positive Wassermann.

[4] Excluding thirteen cases under observation for this period, but treated with more salvarsan for positive Wassermann or clinical relapse.

[5] Excluding one case under observation for this period, but treated with more salvarsan for positive Wassermann.

A rough idea of the probable reduction of inefficiency from syphilis which the routine use of salvarsan is likely to effect may be gathered from Table V which shows that under salvarsan treat-

ment a syphilitic soldier is inefficient in the first year of his disease for thirty-three days, while under exclusively mercurial treatment he loses at least sixty-one days.

TABLE IV.—Wassermann Reactions given by Cases of Syphilis at Different Stages of their Treatment with Mercurial Injections.

Tested by the Original Method

Tested at end of	Total cases	Positive	Negative	Per cent positive
1st course of 6—9 injections	92	68	24	73·9
1st interval of 6—8 weeks	22	16	6	72·7
2nd course of 4—6 injections	81	43	38	53·0
2nd interval of 2—3 months	29	20	9	68·9
3rd course of 4 injections	83	44	39	53·0
3rd interval of 2—3 months	36	20	16	55·5
4th course of 4 injections	124	43	81	34·6
4th interval of 4—6 months	42	24	18	57·1
5th course of 4 injections	67	24	43	35·8
5th interval of 4 weeks	61	16	45	26·2
6th or 7th course of 4 injections ..	115	43	72	37·3
3 months after end of usual period of treatment	289	123	166	42·5

TABLE V.—Showing the Number of Days lost by a Syphilitic Soldier in Hospital and attending as Out-Patient during the First Year of his Disease.

	Days in hospital on first admission	Days in hospital on first re-admission for relapse	Attendances as out-patients assuming no relapse	Extra attendances as out-patient on account of relapses or positive Wassermann reaction	Totals
Mercury	27·9	9·1[1]	25	Not calculated	62
Salvarsan, intravenous, alone or combined with nine mercurial injections	21·5	0·25[2]	10	1·7[3]	33·45

[1] Calculated on assumption that 42 per cent of cases require re-admission at least once and then spend an average of 21·7 days in hospital.

[2] Calculated from Table I. Relapses which occurred in cases observed for twelve months and over, and required re-admission to hospital. Days in hospital on re-admission, 17.

[3] Calculated on basis of a combined course being administered, necessitating nine extra attendances.

That this is not overstating the case in favour of salvarsan is shown by the following facts in connection with this table. The figures under mercurial treatment take no account of second or

third re-admissions for relapse. The annual return of the Military Hospital, Rochester Row, for 1909, showed that 11·9 per cent of syphilis cases were re-admitted twice, and 2 per cent on three or more occasions during the first year. On the other hand, no case treated with salvarsan has yet been re-admitted more than once. No account has been taken of extra attendances made necessary by relapses under mercurial treatment which do not require re-admission to hospital. Of mercurial cases 48 per cent would require to attend more frequently on this account during the first year. Lastly, the relapses shown in the salvarsan series happened under *all* the methods of administering salvarsan excepting the intramuscular and subcutaneous which we have tried, and we now know that some of these are not so efficient as the method we now recommend.

The practical effect of adopting a routine salvarsan treatment of syphilis is illustrated in the following table which shows the average number of syphilis cases (excluding police and transfers from other stations) in the Military Hospital, Rochester Row, during the months of June and July, 1909, 1910, 1911 and 1912, and the number of admissions for syphilis during the same periods. The garrison remained practically constant during the years covered by this table, and we must attribute the reduction which is shown here to the shorter time spent in hospital on first admission, and the smaller number of re-admissions for relapse. The reduction in the work of the out-patient department is shown by the fact that in the year ending June 30, 1910, the number of mercurial injections administered was 4,006; while in the year ending June 30, 1912, it was 2,058. Many cases were still under mercurial treatment during the latter period so that we can expect a greater reduction in future.

TABLE SHOWING THE AVERAGE NUMBER OF PATIENTS IN HOSPITAL, EXCLUDING POLICE AND TRANSFERS, WHO WERE SUFFERING FROM SYPHILIS, AS WELL AS THE NUMBERS OF ADMISSIONS FOR SYPHILIS IN JUNE AND JULY, 1909, 1910, 1911, 1912.

	1909		1910		1911[1]		1912	
	June	July	June	July	June	July	June	July
Numbers in hospital ..	35	42	46	39	31	33	15	9[2]
Admissions	24	22	30	21	22	16	12	9[2]

[1] Many cases were still under exclusively mercurial treatment at this time.

[2] Excluding one case of venereal sore under observation, no spirochætes found. Wassermann negative.

The following cases in which a reinfection has occurred will further illustrate the curative effect of salvarsan:—

Case 1.—Private McQ. December 14, 1910: Admitted to hospital. Early secondary symptoms. Wassermann reaction positive. *S. pallida* in chancre. December 16, 1910: Injection, 0·4 grm. salvarsan intravenously. December 30: Injection, 0·5 grm. salvarsan intravenously. January 2, 1911: No active signs. January 6: Discharged hospital. January 20, February 16, March 16, and June 14: Wassermann reaction negative. September 28: Admitted to hospital with another chancre on a different site. *S. pallida* found. October 5 and 29: Wassermann reaction positive.

Case 2.—Private L. January 10, 1911: Admitted to hospital. Primary chancre and general adenitis. *S. pallida* discovered. January 10 and 23: Injections, 0·6 grm. salvarsan intravenously. February 24, May 4, and September 27: Wassermann reaction negative. February 1, 1912: Readmitted hospital. Fresh primary chancre on another site on penis, numerous condylomata between toes, mucous patches on tonsils, general adenitis, &c. Wassermann reaction positive.

Case 3.—Private B. February 7, 1911: Admitted hospital. Florid secondary symptoms—chancre, adenitis, pustular rash, ulceration of tonsils and condylomata. Wassermann reaction positive. *S. pallida* discovered in chancre. February 9: Intravenous injection of 0·6 grm. "606." February 23, March 9 and March 23: Injections of 0·2 grm. "606." April 1: Discharged hospital. May 2, July 6, September 19, and January 10, 1912: Wassermann reaction negative. March 4, 1912: Readmitted hospital with a fresh chancre on different site. *S. pallida* discovered, papular rash, adenitis. Wassermann reaction positive.

Case 4.—Lance-corporal M. March 31, 1911: Admitted to hospital. Primary chancre in which *S. pallida* discovered. Wassermann reaction positive. April 1: Injection of 0·6 grm. "606" intravenously. April 15, April 29, May 13: Injections of 0·3 grm. "606" intravenously. June 22: Readmitted with fresh chancre on penis, different site. *S. pallida* discovered. Wassermann reaction positive.

Case 5.—Lance-corporal S. May 1, 1911. Admitted to hospital. Primary chancre only, in which *S. pallida* was discovered. Wassermann reaction positive. May 4: Injection, 0·6 grm. "606" intravenously. May 17, June 1, and June 16: Injection of 0·3 grm. "606." September 19: Wassermann reaction negative. November 24: Development of fresh chancre in another situation. *S. pallida* discovered.

All of the above cases were treated with salvarsan for the reinfection. In all the symptoms rapidly cleared up and the Wassermann reaction again became negative. None of them have shown any further signs of syphilis. With the exception of Case 4, which passed out of observation after two months, all have been followed up to the date of writing.

Whether we accept re-infection as evidence of absolute cure of the first attack, or not, we are bound to admit that it indicates

a complete suppression of the *S. pallida* in the tissues, a suppression which was seldom secured with the best mercurial treatment. C. F. Marshall has stated that in order to establish the fact of re-infection it is necessary for two distinct attacks of primary and secondary to have been seen in the same patient by the same observer. The same author has suggested that some of the cases of supposed re-infection which have been reported after salvarsan treatment may have been suffering from chancriform gummata. We do not think it necessary for the patient to have suffered from two attacks of secondary syphilis to prove re-infection. This may have been necessary in the days when the diagnosis of a primary sore rested solely on the uncertain ground of clinical evidence, but now it is sufficient evidence of the first attack that *S. pallida* has been demonstrated in the sore, and we think that re-infection is proved if the second sore occurs at a reasonable interval from the first, is on a different site, has the clinical appearance of a primary sore and contains *S. pallida* in large numbers. As for the suggestion that these new sores on the penis may be chancriform gummata, we can only say that such lesions seem to have been reported in extraordinary numbers since salvarsan was discovered, while *S. pallida* is not usually found in such large numbers in gummata as we found in the sores which we have described as examples of re-infection.

Good as these results of salvarsan treatment are we have reason to believe that better will be obtained in future by using mercurial injections in conjunction with salvarsan, instead of relying exclusively on salvarsan. In Tables I, II, and III we have divided our salvarsan cases into three groups: (1) Those treated with a single subcutaneous or intramuscular injection of salvarsan; (2) those to whom we administered one or more intravenous injections; and (3) those who were treated with an initial intravenous injection of 0·6 grm. salvarsan, then nine intramuscular injections of mercurial cream at weekly intervals, and, lastly, an intravenous injection of 0·6 grm. salvarsan. A comparison of the relapses under each of these groups will show that while a subcutaneous injection is not nearly so permanent in its effects as one or more intravenous, the results obtained by either of these methods are easily surpassed by those which follow the combined course of mercury and salvarsan we have described.

We may say that we are treating a series of cases with three fortnightly intravenous injections of salvarsan and four injections of calomel cream, the idea being to see if the initial course of

treatment can be made shorter. So far, however, the results have not been so good with regard to the Wassermann reaction, and for the present we recommend the combined course of salvarsan and mercurial cream injections as the one which appears most likely to give the best results.

In a previous paper we have mentioned the great importance of commencing treatment in the primary stage of the disease; if possible, before there is any induration of the sore or the Wassermann reaction has become positive. Reference to Tables I and III will show that the primary cases relapsed less frequently and gave a lower percentage of positive Wassermann reactions at different periods than the group comprised of all the fresh cases we have treated with salvarsan. Further, out of 101 primary cases treated with salvarsan and under observation for three months or longer only two have developed secondaries. It should also be mentioned that five out of the nine primary cases which subsequently gave a positive Wassermann reaction were positive before treatment was commenced. The four cases which were negative beforehand were treated, one with a single subcutaneous injection and three with four intravenous injections, neither being methods of treatment we would now recommend. It is clear from these results that it is well worth while to use every effort in encouraging men to report sick early and in making the diagnosis with the least possible delay.

The question of dosage of salvarsan is one which has lately arisen in connection with certain fatalities after salvarsan injections. Regarding the subject of deaths, we may say at once that in forty-three subcutaneous or intramuscular and 1,613 intravenous injections we have not experienced any untoward incident, while Wechselmann states that, in the course of over 12,000 injections, he has not had any death which could be attributed to salvarsan. When we consider that probably more than a million injections must have been given all over the world, fatalities amounting even to a fraction per thousand of the injections could not have been concealed and would have filled a prominent place in the literature. Dreyfus has collected and analysed the records of 150 deaths after salvarsan. After eliminating those in which the death was either due to faulty technique, or was a coincidence, or followed gross disregard of well-known contra-indications, about a dozen were left which could be attributed to salvarsan poisoning. Excluding, again, those which were due to decomposition of the drug at the site of an intramuscular injection,

a few remain which have been reported by Marschalkó and Vesprémi, Queyrat, Lesser, Kannengiesser, McDonnell, and others, and are not so easy to explain. The symptoms and post-mortem appearances presented by these cases subsequently to the injection were very similar and indicated a common factor in their causation. Briefly, a few days after an injection there ensued epileptiform convulsions, then coma, and death on the third to the fifth day, and the autopsy showed punctiform hæmorrhages in the brain and basal ganglia.

Professor Ehrlich, noting that these fatalities have almost always occurred in patients who were suffering from early generalized syphilis, believes that they are due to the liberation of endotoxins from spirochætes in the brain. On this account he recommends that in early generalized syphilis—that is, after the sore has indurated, the inguinal glands enlarged and the Wassermann reaction become positive—the treatment with salvarsan should proceed very cautiously. His directions are, first, to administer two intramuscular injections of calomel, then 0·1 grm. salvarsan, then two more injections of calomel, and, a few days later, 0·15 grm. salvarsan. Only when it is seen that this is well borne would he increase to a full dose. In cases of early primary syphilis, on the other hand, he recommends a full initial dose of salvarsan and intensive treatment from the commencement with a view to aborting the disease.

For reasons which we may shortly mention, we have found some difficulty in accepting Ehrlich's explanation of these deaths. It is likely that, whatever their immediate cause, most of the fatalities would fall into the group of early secondary cases since this must comprise the vast majority of cases treated with salvarsan. But not all the deaths of this kind have occurred in early secondary cases. Lesser reports a similar death in a patient whose disease was of fifteen years standing, while Marschalkó and Vesprémi report one in which the patient had contracted the disease twenty years previously and was apparently in good health. Again, if death were due to liberation of endotoxins one would expect it to occur after the first dose when, presumably, the greatest number of spirochætes are destroyed. In the majority of these cases, however, the fatality occurred after a second injection administered within a week or eight days of the first.

It seems to us reasonable to suppose that, apart from the fact that, as Yakimoff has shown, the toxicity of salvarsan may be increased by the use of impure distilled water and salt solution,

some patients may be very exceptionally susceptible to salvarsan, however carefully prepared. This susceptibility may possibly be increased in the early secondary stage, and is certainly increased by such indiscretions as violent exercise, alcoholic excess and railway travelling a few hours after the injection—a matter of some importance to those responsible for the after-treatment of salvarsan cases.

It is especially noteworthy that most of these deaths have followed the second of two injections which was given at an interval of eight days or less, and it is possible that the drug has exercised a cumulative action in these cases.

Marschalkó and Vesprémi claim to have produced the same symptoms and post-mortem appearances in rabbits by overdosing them with salvarsan.

The true explanation of these fatalities is important, because if we believe with Ehrlich that they are due to the liberation of endotoxins we should proceed fearlessly to treat our early primary cases with full doses of 0·6 grm. salvarsan, but would exercise considerable caution when the disease has become generalized. If, on the other hand, the cause lies in exceptional idiosyncrasy the routine procedure would be much easier. We should not let our patients commit any of the indiscretions we have mentioned, nor repeat the injection at such a short interval as to cause any cumulative action, and we should try to ascertain if the maximum effect of one intravenous injection can be achieved with a smaller dose than 0·6 grm. We do not think the last is nearly so important as the first two precautions we have mentioned. We have not been able to find accounts of more than three cases where a patient in apparently good health died after an initial dose of 0·6 grm. salvarsan which was properly administered, and we believe that, if care be taken not to repeat the injection too quickly, if punctilious regard be paid to details of technique (asepsis, freshly distilled water, freshly prepared solutions, &c.), as well as to after-treatment, and if the contra-indications are properly considered, the risk of death after such a dose is infinitesimal, whether it is administered in the early primary or the early secondary stage of syphilis.

Some attention is due, however, to those workers who claim as good results from the injection of 0·3 grm. as those obtained by workers using the larger dose. In a previous paper we have expressed the view that, considering the rapid beneficial effect which follows an intramuscular injection and the well-known fact that absorption of the remedy from the site of such an injection

is very slow, it must require a very minute dose of salvarsan in the circulation to destroy all the spirochætes which can be reached by this means.

In the same paper we agreed with the opinions of those who consider that relapses are caused by spirochætes which at

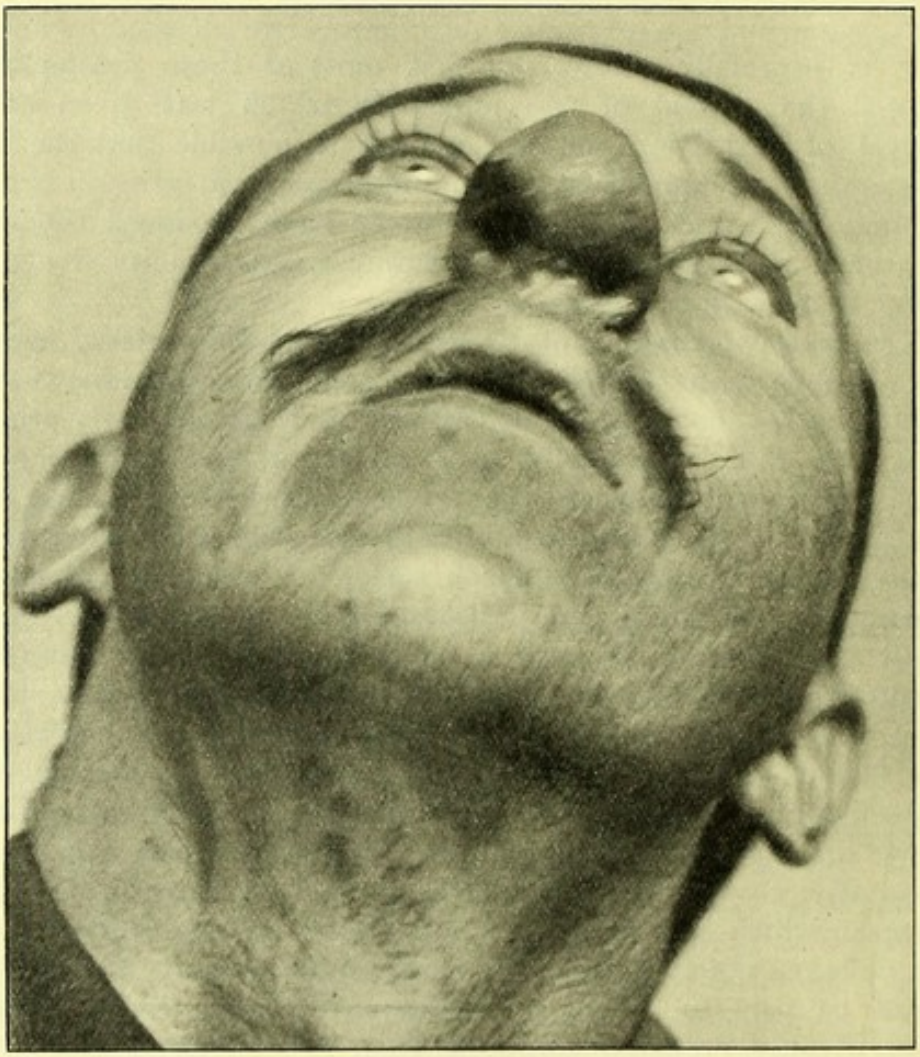

FIG. 1.—Syphilitic ulceration of nose of one year's duration. Contracted syphilis four years ago. Regular mercurial and iodide treatment with tonics.

the commencement of treatment were buried in thrombosed vessels and sclerosed areas and consequently remained inaccessible to circulating fluids until after the first dose of salvarsan had been excreted. If this be true it is possible that the administration of 0·6 grm. in any one injection may effect no more

than would 0·3 grm. To borrow an example from bacteriological technique, when we sterilize by the intermittent method, one hour's steaming on any one day is no more effective than steaming for half that time, nor does it save us from the necessity of repeating the operation on two more days. In order to test this possibility we have lately commenced a series of cases to

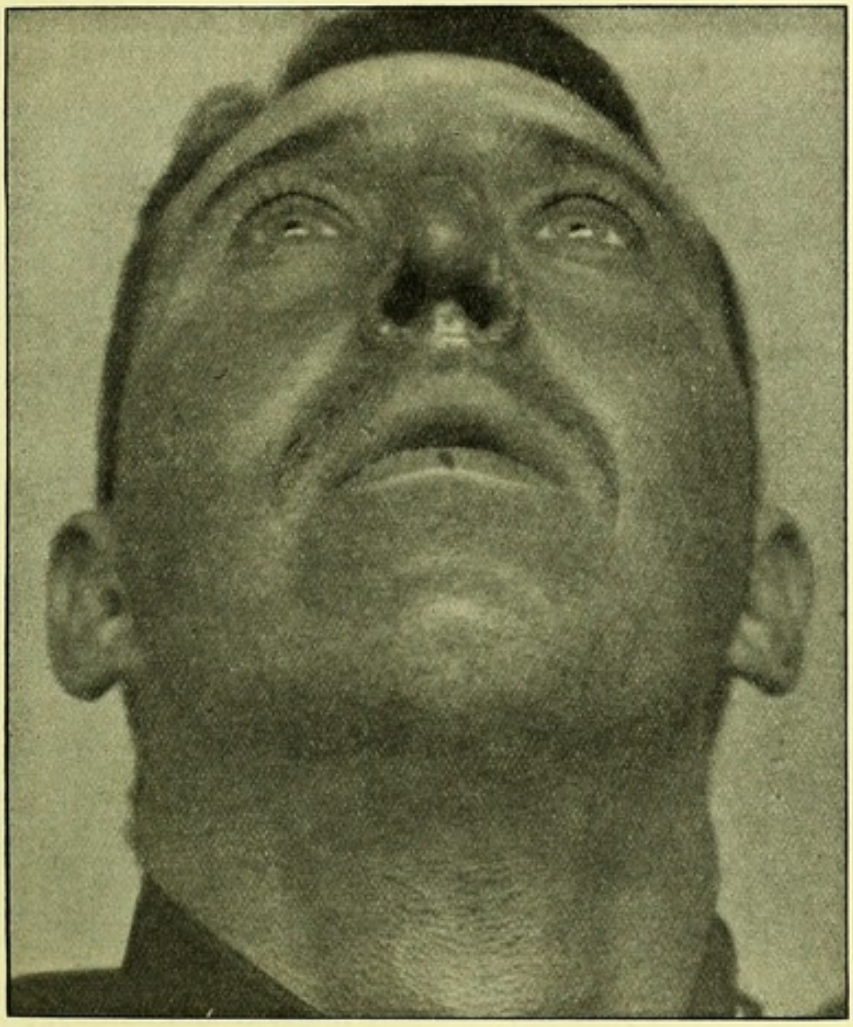

FIG. 1A.—Same case as 1, thirteen days after commencing neosalvarsan treatment. Treated with 0·9 grm. neosalvarsan, two days' interval; 0·9 grm., eleven days' interval; and, lastly, 1·3 grm.

whom we are administering three injections of 0·3 grm. salvarsan in four weeks and four calomel injections in the same month, and intend to compare the results with those obtained in a series of fifty cases which we have treated similarly, except that the dose of salvarsan has been 0·6 grm.

Neosalvarsan.

Professor Ehrlich very kindly gave us a generous supply of neosalvarsan, or "914," and we have treated a number of cases with it. The new preparation is very readily soluble in water, or, as Ehrlich recommends, in 0·4 per cent sodium chloride solution, in which it forms a neutral solution, and is ready for injection as soon as it has dissolved. It is not so stable as salvarsan, being converted into a very toxic compound if dissolved

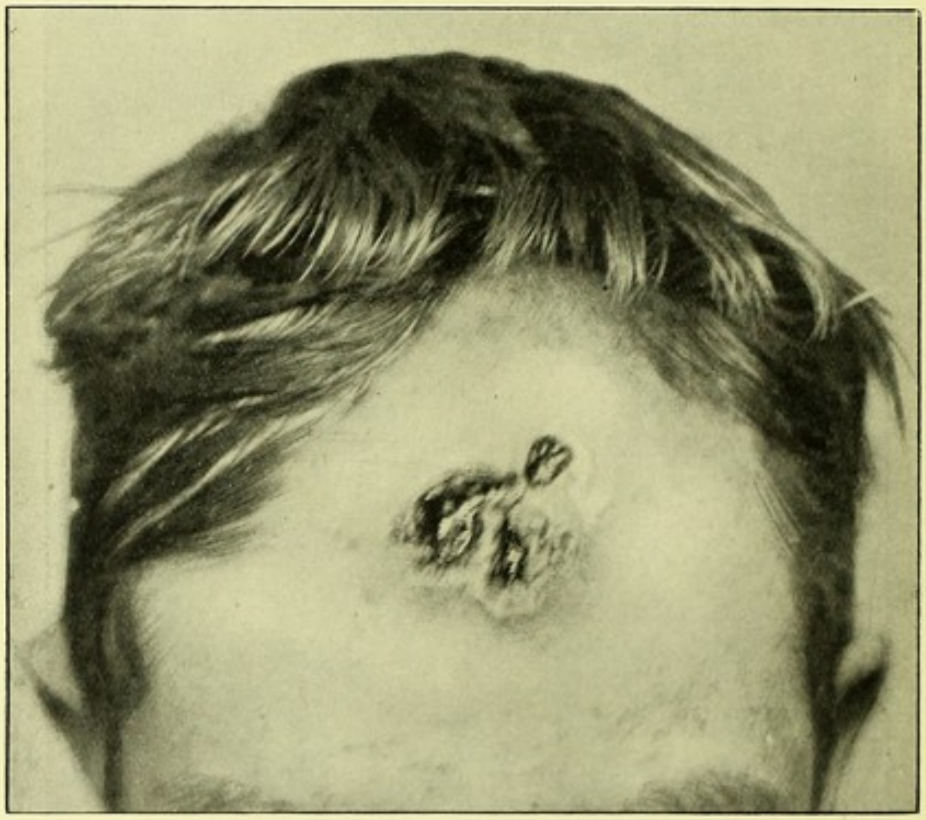

Fig. 2.—Gumma of frontal bone, with ulceration of six months' duration. Contracted syphilis four years ago. Energetic treatment with mercury, potassium iodide and iodipin.

in water which is above 80° F., or in physiological salt solution, or if unduly exposed to air. It should therefore be dissolved and administered in water, or 0·4 per cent salt solution, at room temperature and injected at once.

In administering neosalvarsan our technique has been as follows: Using the apparatus we employ for salvarsan, the procedure is the same up to the point where everything is ready

for the remedy to be poured into one of the funnels, except that distilled water is used instead of physiological salt solution. The patient is then put on the table, and the skin over the site of the proposed puncture sterilized. Not till then is the tube of neosalvarsan opened and its contents dissolved in distilled water (0·9 grm. in 150 c.c.) at room temperature. The remedy dissolves at once and is poured into the funnel prepared for it. In order to avoid having to inject so much distilled water as is contained in

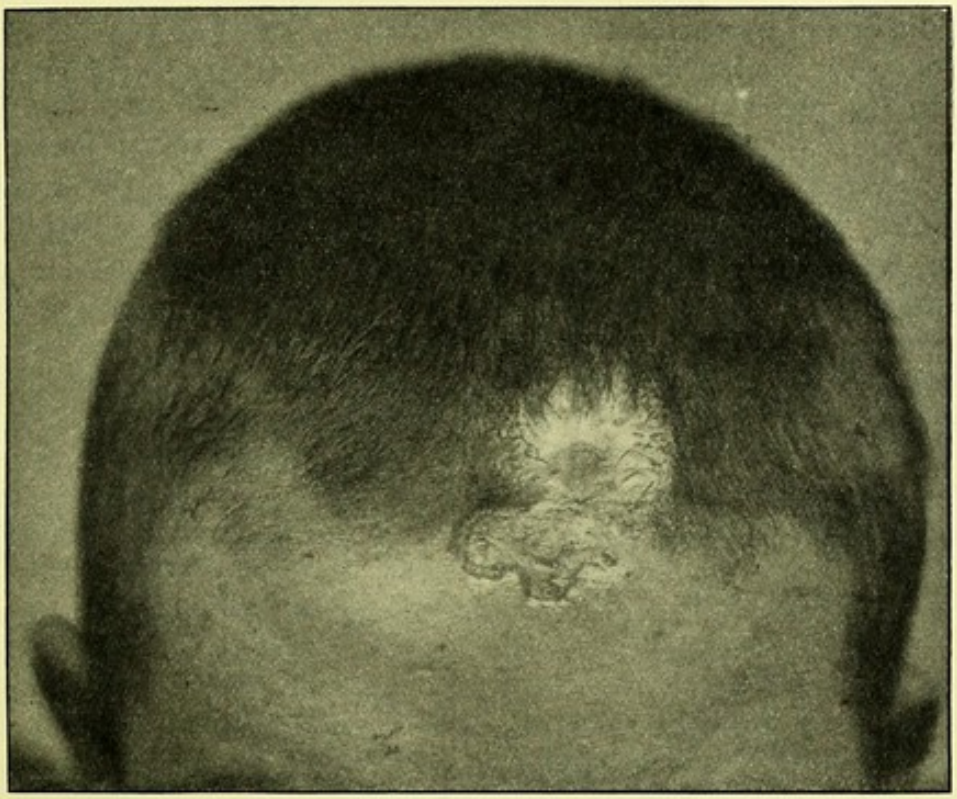

FIG. 2A.—Same case as 2, after neosalvarsan treatment. Treated with 0·9 grm. eight days' interval ; 1·2 grm., seven days' interval ; and, lastly, 0·9 grm. neosalvarsan.

the rubber tubing between the funnel and the needle, before puncturing the vein the clip which controls the tube leading from the neosalvarsan is opened, and the distilled water in the rubber tubing is run off till the neosalvarsan solution has reached the lowest glass window. The technique is then the same as in administering salvarsan. We have not ventured to administer such enormous doses or to repeat the injection after such short intervals as Schreiber and others have done, and the largest amount we have given has been three doses of 1·2 grm. at weekly intervals. Since

3 of neosalvarsan is equivalent to 2 of salvarsan this would correspond to three doses of 0·8 grm. salvarsan. Ehrlich now recommends that workers should not administer neosalvarsan in doses any larger than those which correspond to safe amounts of salvarsan, nor any more frequently. Reactions are much less frequent after neosalvarsan than after the older preparation. The therapeutic effect of neosalvarsan seems to be much the same as that of salvarsan, as will be seen from the illustrations, but it is much too early to speak of its permanence. The chief disadvantage of neosalvarsan is its instability; its advantages are that it is very quickly prepared and causes reactions less frequently than the older preparation.

We are indebted to Professor Ehrlich for kindly giving us a supply of neosalvarsan and for his very valuable advice on many points. We would also like to record here our appreciation of the help we have received from numerous brother officers who supplied us with monthly reports on the progress of many of our cases and from Mr. Gibbs, Royal Army Medical College, to whose photographic skill we are indebted for the illustrations in this article.

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THE VACCINE TREATMENT OF GONORRHŒA, WITH NOTES ON THIRTY CASES.

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The literature on the subject of gonorrhœal vaccination is so contradictory that a trial of this method of treatment was decided on in December, 1910. The various reports on vaccines were consulted, and the following résumé was compiled.

The dosage of vaccine has varied from 500,000 to 1,000,000,000 gonococci in acute and chronic disease respectively. Each bacteriologist prepared the vaccine in his own way, so that there was only an approximate relation between the immunizing power of similar doses in any two vaccines.

The following record of some 300 cases treated by gonococcal vaccine has been extracted from literature.

Hale White and Eyre [1] treated four cases of chronic gonorrhœal arthritis which were unaffected by local or general medication. Opsonic control was employed. In two of the cases four to ten million cocci were injected every seven days; this resulted in cure. Another case had an autogenous vaccine, of which he was given 5 to 25 million cocci with six days interval between the injections. The fourth also had an autogenous vaccine; 100 to 200 million cocci were injected, with complete recovery in five weeks.

Eyre and Stewart [2] published their results in fifty-three cases. The gonococcus was isolated and grown on blood agar. The twenty-four-hour-old culture was sterilized at 50° C. for one hour, and again heated at the same temperature for half an hour, after the addition of ½ per cent trikresol. Three to ten strains were used. After the first few injections, which contained as many as 100 and 250 million cocci, they did not give a higher dose than 25 million cocci.

In acute gonorrhœa, they usually employed a dose of 5 million cocci; this was followed by a negative phase lasting three to four days, after which a strong positive reaction set in. They advise daily opsonic index observations. If the index is variable, they recommend a dose of ½ to 1 million cocci, if it is steady 2 to 5 million cocci are injected. In chronic infections small injections were found to be more beneficial than large ones; the best results were obtained with 1 to 2 million cocci, every three to five days, or 3 to 5 million

every five to seven days, or 10 million with an interval of eight to ten days. In orchitis a small dose is best.

Hartwell [3] in discussing gonorrhœal arthritis, states that of thirty-one subacute cases, twenty-seven made perfect recoveries. In chronic affections vaccines are not of much benefit, especially if ankylosis is present. His vaccine is sterilized at 60° C. for one hour, or kept on ice all night, then lysol added and allowed to act on the organisms for twelve hours. The strength of the lysol is 0·25 per cent. The author did not find any difference between the heated and unheated cultures. The most efficient initial dose for acute arthritis was 10 to 25 million cocci; for chronic cases as many as 600 million cocci have been injected. The interval between inoculations was two to four days in acute cases, and five to seven in the more chronic affections.

Aronstam [4] treated fifty-four patients with stock vaccines, and no local applications. The average time under treatment was three weeks. Three to eight injections were required to effect a cure. He summarizes the results of his experience by stating that recovery from acute gonorrhœa takes place in four weeks, without injections or irrigation, that vaccines act well in epididymitis, and prostatitis. He also observed that latent arthritis became manifest under the vaccine treatment, but was eventually relieved.

Ballinger [5] made use of a mixed vaccine in mixed infections, and a stock culture in uncomplicated gonorrhœa. In addition he had recourse to massage, injections, and irrigation. He considers massage of joints at the same time as vaccination inadvisable, owing to the liability of causing a negative phase. In acute cases he gave from 5 to 10 million cocci, in chronic cases up to 50 million cocci, beginning with a dose of 15 million cocci, and allowing five to eight days' interval between the injections. His conclusions are favourable, if vaccination is only looked on as an adjuvant to local treatment.

Dieulafoy [6] describes the course of a case of gonorrhœal septicæmia, with typhoid symptoms which recovered. He made a culture from the blood in peptone ascitic broth, obtaining a pure culture of the organism in thirty hours. He gave a vaccine containing 5 millions of this organism, after which the temperature dropped to normal. Three days later the same dose was repeated with marked benefit. A third injection of 10 millions was followed by complete recovery.

Carl Mianini [7] reported *in extenso* four cases of arthritis, and showed the interdependence between the opsonic index and the size

of the dose. The first patient had 2 million gonococci injected. His opsonic index before the injection was 0·84 ; after the injection it fell to 0·42 ; the fall was accompanied by an increase in all the symptoms. The second dose was reduced to 1 million. Six days later the index was 0·83, and he appeared much better. The joint pains returned after a week's freedom from symptoms, and twenty-six hours after an injection of 200 million cocci again disappeared. A relapse of pain on the seventh day was treated by an injection of 300 million cocci ; pain left the joints that evening. His opsonic index at the end of the course was 1·23. The second case received a first injection of 30 million cocci, as he had an index of 0·96 ; this then fell to 0·73. In five days the opsonic index had risen to 1·42. In all, five doses gradually increased to 350 million cocci were given. The third case had an initial dose of 4 million cocci, followed at intervals of three, five, six, and eighteen days respectively, by 30, 100, 150 and 200 million cocci. The opsonic index varied after the doses thus : at first the index was 0·96 ; this fell to 0·47. The next index was calculated as 0·54 ; five days later it was 0·89. On the fourteenth day it was 1·28, and on the thirty-second day it had fallen to 0·68.

Case 4 had an index of 1·12 before the first injection, which contained 250 million cocci. Next day all pain had ceased. On the third day 100 million cocci were given and the opsonic index was 0·78. The daily record showed a lower index each day and an increase in the pains. Two days later 400 million cocci were injected ; this was followed by a rise in the opsonic index to 1·04. Seven days after the last injection the patient received another 400 million cocci, and his opsonic index fell to 0·47. There was no relapse from this time, and massage was resorted to.

In conclusion the author is of opinion that the loss of pain is not to be taken as an index of the cure of the disease ; it only shows that gonococcal vaccine has a specific action on gonorrhœal arthritis.

Thomas [8] gives three years' experience of specific immunity. He describes the treatment of gonorrhœal vulvo-vaginitis in children, and shows brilliant results from injections of 5 million gonococci. Four joint cases were either cured or improved with a dosage of 50 million gonococci. Autogenous vaccines have given the best results in his hands. Finally, the author states that vaccination is only an adjuvant to local treatment.

Irons [9] made his vaccine from organisms heated to 60° F. for one hour. When a dose of 500 million cocci was experimented

with, the reaction started within twelve hours and persisted for twenty-four hours. With 50 million cocci there was little or no reactive response. In summarizing his results, he comes to the conclusion that the action of vaccine therapy is better in the case of infected joints than in any other form of gonococcal infection.

W. Friedlander and Reiter [10] in discussing the various complications of gonorrhœa say that in acute, subacute and chronic epididymitis, vaccines give the best result. Reiter's preparation was used in doses of 0·2, 0·3, 0·4, 0·6, 0·8 and 1 c.c., each cubic centimetre containing 5 million gonococci. The interval between injections was three days. Infiltration disappeared very rapidly, leaving only a small nodule. Deep lesions appeared to heal before the original focus in the mucous membrane; the urethral discharge remained unchanged.

In acute prostatitis improvement was not noticed. However, in acute and subacute follicular prostatitis both the objective and subjective signs of the disease were quickly ameliorated. Spermocystitis was improved, but not cured. Infection of the ducts of Bartholin's glands was not influenced by vaccination.

Allen [11] has come to the conclusion that in gonorrhœal infections phagocytosis and opsonin formation is not of as much importance as in some of the other vaccine-treated diseases. In support of this, he mentions that two cases of gonorrhœa with equally numerous cocci in the pus leucocytes, give widely different results in culture ; thus, one may produce a poor growth, the other an abundant one. He has had very encouraging success in acute disease. As an initial dose, the author recommends 25 million cocci, followed by a similar dose ; later doses may be increased up to 50 million cocci. In addition a mild antiseptic irrigation should be carried out at the same time. If the cure is not complete in two or three weeks, as is usual in his cases, the injections are increased, even as many as 1,000 million cocci being given. No secondary complications were met with and he says that in all cases the vaccine should be increased till good results follow.

In reviewing the foregoing work done by various authors the following conclusions have been arrived at. There is unanimity of opinion that vaccine therapy is an advance on the ordinary medicinal method. There is also an agreement on the necessity of high dosage in arthritis of gonorrhœal origin. Acute gonorrhœa may be cured if treated with irrigation; massage of the prostate is required in posterior urethritis, and sounds in the more chronic disease, with or without internal

treatment. The average time in which recovery can be assured is six weeks, provided that the patient begins his course of irrigation, &c., in the very earliest days of his attack. Both Aronstam and Allen have succeeded in curing urethritis with vaccines in three weeks, the former without any other aid, the latter with mild antiseptic irrigation. On the other hand, most of the authors who have tried inoculation for uncomplicated gonorrhœa state that there is not much advantage to be gained therefrom. The general consensus of opinion appears to be that the deeper the lesion the more active is the immunizing power of gonococcal vaccine.

Preparation of Vaccine.—In an article by Martin [12] in the *Journal of the Pathological Society*, on the growth of the gonococcus, he describes a culture medium which has given excellent results in his hands.

Following his procedure a medium was made containing:—

Beef extract broth, to which was added
Disodium phosphate 0·5 per cent,
Witte's peptone 1 per cent,
Agar agar 2 per cent.

When sterilized in test tubes the medium is "slanted" and kept till required. A patient with a gonorrhœal discharge from his urethra, from whom it is desired to make a gonococcal culture, is brought to the pathological laboratory. The glans penis is cleaned with absolute alcohol, and allowed to dry. Two tubes of the medium have the surface of the agar evenly smeared over with about four drops of sterile hydrocele, ascitic or pleuritic fluid. The tubes are now placed in the incubator until they become heated to 37° C. The first part of the urethral discharge is removed from the tip of the penis; two loopfuls of the deeper discharge at the meatal orifice are taken on a platinum needle and quickly transferred to the surface of the agar slope which is marked, and returned to the incubator. A pure culture, or at least isolated colonies, can be subcultured on a similar medium. A large percentage of excellent growths of the gonococcus in pure culture was obtained in this way.

A twenty-four-hour-old culture in 1 per cent sodium chloride is sterilized by heat applied for an hour at 59° C. The vaccine is diluted till 5 minims contain 20 million gonococci. At first three cultures from three cases were mixed, but at a later period, and when better results were got by culture, ten different cultures entered into the vaccine. To keep the vaccine sterile, ½ per cent

lysol was added to the stock, which was stored in rubber capped bottles, 20 c.c. in each.

Of the thirty cases, twelve had a vaccine procured from one of the large manufacturers, the remainder had a vaccine made from a case of gonorrhœa acquired locally as detailed above.

In beginning the systematic vaccination of the thirty cases our idea was to investigate whether the opsonic index could be dispensed with, and whether vaccine inoculation for gonorrhœa could be made a simple clinical method devoid of dangers and capable of employment without the aid of laboratory technique. Gonorrhœa is here meant to include acute, chronic, and blood-stream infections. No attempt was made to select cases. All cases which were admitted into the Royal Infirmary, Dublin, during the period were treated in the same way, with the exception of some cases of mixed gonococcal and other organisms; in these cases cultures of the *Bacillus acidi lactici* were injected into the urethra.

Case 1 had been in hospital since September 5, 1910. He had two or three exacerbations of the disease during October and November. Examination by the urethroscope showed a condition of soft infiltration along the whole of the urethra. A pure culture of gonococcus was isolated from the deep urethra. The first dose of gonococcal stock vaccine was injected on November 29, 1910, dose 5 million cocci. Four days later 50 million cocci were given, and no reaction followed. In forty-eight hours the urethral discharge had ceased for the first time since early in November. The third injection was given on December 10, 1910, as a return of the discharge occurred with threads in the "anterior" urine glass (Thompson's two-glass test). On December 17, 1910, these threads contained a bacillus, but no gonococcus. A culture on ascitic agar of one of these threads produced a diphtheroid organism, and a grey non-liquefying coccus. The *B. acidi lactici* from one of the proprietary tablets "Saurin" was grown on 1 per cent extract of malt in agar. The pure culture incubated for twenty-four hours on the medium just mentioned was emulsified in salt solution and injected into the urethra by means of Ultzmann's instillation syringe. This was followed by an increase of pus for twenty-four hours, and the threads disappeared. The injection was repeated three days later, and an irrigation of permanganate of potash was given next morning. Threads and discharge from the urethra ceased and no further signs of gonorrhœa have recurred.

This patient was one hundred and sixteen days under treatment, but was only forty-five days under vaccine therapy. The gonococcus alone was the pathological factor for ninety-six days, the secondary infection only occurred in the last three weeks.

B. acidi lactici has been the object of some investigations by Cannata and Mitra [13], as to its action on various pathological organisms. The point of interest as regards this paper which was elicited by them, is that the *B. acidi lactici* has a decided bactericidal action on organisms of the typhoid, paratyphoid, and dysentery groups, and also on the staphylococci; while the *B. coli* group, *B. pyocyaneus* and *B. prodigiosus* are practically unaffected by contact with the organisms of the lactic acid family.

Case 2. The next case, also treated by *B. acidi lactici* in addition to vaccines, is of interest owing to the unusually numerous gonococci in the urethral discharge, and the copious growth on the disodium phosphate pleuritic fluid agar. Though the diplococci looked like gonococci microscopically, and were also Gram negative, the appearance on the medium was more like a rather transparent culture of *Staphylococcus albus*. However, no growth could be obtained on ordinary agar, and subcultures on the special medium gave positive results. Three weeks irrigation with permanganate of potash solution did little more than lessen the amount of discharge, but made no impression on the number of gonococci.

To test the effect of *B. acidi lactici* on the gonococci an emulsion of the bacillus similarly prepared to that used in the last case was injected on March 4, 1911; no change was noticed in the urethral discharge, or in the infecting organism.

On March 6, 1911, a second injection was given of *B. acidi lactici;* this was followed by a copious discharge of pus, much pain in the prostate, in fact an acute attack of prostatitis. Two days later permanganate of potash irrigation was resumed. The "local" stock vaccine had now been prepared, and a dose of 50 million was given on March 13, 1911. No reaction followed. Doses of 20 million were injected with weekly intervals. A slight reaction followed the fourth vaccination. At this time the discharge had disappeared and only a slight "haze" remained in the urine, but prostatic pain and frequent micturition were still present. The dosage of gonococci was increased in the following weeks to 35, 40 and finally 100 million; the patient had a slight local reaction after each inoculation; irrigation was continued all through this period. The prostate did not improve to any great extent until sounds and massage were resorted to, when the change was at

once marked. This case was ninety-six days under treatment and neither vaccine or *B. acidi lactici* had a curative effect.

Case 3 had a pure gonococcal infection and a pure culture of the gonococcus was isolated. Potassium permanganate irrigation was begun on admission, but as the urethral discharge did not clear up rapidly another trial was given to the *B. acidi lactici.* One urethral injection was employed on March 4, 1911, on which date gonococci were found in the discharge. Two days later no discharge could be expressed, and a second syringeful of bacilli was introduced high up in the urethra. A third dose was given on the following day. No unpleasant effects followed these injections, and irrigation with Condy's fluid caused the disappearance of the introduced organism immediately. On March 10, 1911, only a faint haze could be seen in the urine. Two vaccinations with his own gonococci with a three-day interval completed the cure of this case. The number of days under treatment was twenty-six. Whether one should attribute the rapid cure of this man to the *B. acidi lactici* or not, is a difficult question to answer, but the fact remains that the discharge from his urethra disappeared and gonococci could not be found within forty-eight hours of beginning the bacillary applications.

From Case 4 a pure gonococcal culture was isolated, and grown to many generations. He had two injections of "manufacturer's" vaccine, of 25 and 50 million respectively at an interval of one week; this treatment was followed by an increase of both the discharge and the number of gonococci. Nitrate of silver was substituted for Condy's fluid as a douche for the urethra, with improvement in the urethritis.

On March 4, 1911, an emulsion of *B. acidi lactici* was injected and also on the following day. No discharge was visible after the second dose, until March 18, 1911. A "local" stock gonococcal vaccine had by this date been made, and five doses were given, beginning at 12 million, while the fifth contained 32 million gonococci. There was a slight reaction after the fourth dose in the form of an increase of discharge; no organisms, however, were found. Before this patient was considered clear he was under treatment for eighty-six days, and a really satisfactory result was not obtained, either with the vaccine or with Massol's bacillus.

The next case (No. 5) was one of anterior and posterior urethritis, with papillomata on the glans penis associated with a septic condition of these warts. Owing to the complication of sepsis on a chronic gleet, this is not a fair test of the time required

for treatment by vaccine. The case is mentioned here to show that the lactic acid bacillus has a decided influence on septic processes. Two injections of 50 million cocci, each at fourteen days' interval, had the effect of eliminating gonococci, but not the discharge; the warts were still in a very purulent state in spite of constant irrigation, and organisms of all kinds were found in the pus. One injection of *Bacillus massol* cleared up these organisms, and the urethral discharge ceased within forty-eight hours.

From Case 6 a pure culture of gonococci was isolated. He had a relapse when one month free from signs of the disease. By means of the urethroscope a small ulcer could be located 2½ in. from the meatus. No curative effect followed eight vaccinations at weekly intervals of a stock gonococcus vaccine containing from 5 to 50 million cocci. Late in the disease an injection of *B. acidi lactici* was tried as there was a Gram positive diplococcus in addition to the gonococcus found in the urethra; it had no influence on the course of the urethritis. Eventually, a cure was effected by silver nitrate applied to the ulcer by Guyon's syringe.

These cases are placed together to show the effect of combined irrigation, vaccine, and *B. acidi lactici*. They were on an average eighty-one days under treatment, the shortest was twenty-six days, and the longest 116. It seems as if the living bacillus has some influence in eliminating a septic infection complicating gonorrhœa. Further work is required on this point before a definite answer can be given.

Gonorrhœal Rheumatism.

Three patients were treated for this complication with vaccines. Two of them suffered from a relapse after vaccination, but were finally discharged from hospital quite cured.

Case 7 had urethritis with pain and swelling of both knees and the left shoulder. On the day following admission to hospital he had 50 millions of stock vaccine. His left knee became more swollen and painful. Five days later 10 million were injected without result. The third dose was 100 million, which caused some reaction for thirty-six hours. No irrigation had been tried up to this date, when 1 in 10,000 solution of permanganate of potassium was used to flush out the urethra morning and evening. A fourth injection of 150 million cleared up the joint pains, but slight swelling still remained. Five days later, 150 million were injected; this aggravated the urethral condition and caused the reappearance of pus with numerous organisms in the cells. The rheumatism disappeared on the twenty-

eighth day, and a final vaccination of 200 million gonococci produced no reaction. The urethral discharge and the threads in his urine disappeared on the next day, and did not recur.

Case 8 had been treated for two and a half months by ordinary methods without success, before coming under observation. In addition to urethritis he had both right and left ankles swollen, also the plantar fasciæ were very tender to touch. He received three injections, each containing 50 million cocci, with an interval of seven days between each injection. All signs of the disease disappeared on the fourteenth day after the first dose. However, in two months a relapse of the rheumatic pains and urethritis compelled this patient to return to hospital. At intervals of three days he had the following quantities of vaccine: 70 million, 20 million, and 30 million. On the seventh day of the relapse the urine became clear and he had no urethral discharge. The fourth injection, 40 million, was given on March 23, 1911, as a slight return of pus was found. As no active signs were present, and it was considered that there was no fear of affecting the urethra at this stage, a dose of 200 million was introduced under the skin of the shoulder, on March 30, 1911, in order to eradicate the rheumatism from his ankles. A marked urethral reaction followed in two days, a large amount of blood and pus also appeared; to control the former tannic acid bougies were necessary. However, the blood and pus cleared up in a week. Two further injections of 150 million on April 6 and 11 were not felt in any way by the patient, and he has not had a relapse of rheumatism for one year. He was in hospital thirty-three days, and free from disease in twenty-eight days.

The relapse in this case was due to insufficient dosage, and during the second admission time would appear to have been lost by giving the small and frequent rather than larger doses at longer intervals.

Case 9 was admitted to hospital with an uncomplicated attack of gonorrhœa on April 11, 1911. He had acute anterior and posterior urethritis. Three vaccinations each of 20 million were given with five days interval between them. At the same time irrigation with silver nitrate 1 in 10,000 was employed. No culture was obtainable, as the gonococci were few in number. On May 17, 1911, no signs of the disease were present and the patient was looked on as cured on May 27, 1911. Two months later, however, he was admitted to another hospital with subacute gonorrhœal rheumatism of both ankles, plantar fasciæ and shoulders. A vaccine

was not given and he was treated by other methods for some months with only partial success. In January, 1912, he was re-admitted with the same condition of his ankles and plantar fasciæ as before. Bier's congestion treatment was tried by means of rubber bands and hot air, but with little result. On February 2, 1912, 20 c.c. of antigonococcal serum was introduced under the skin of the abdomen as an experiment. The effect was most unexpected, for within forty-eight hours the patient could hop on either foot without pain or discomfort, and has been well since that time.

Gonorrhœal Epididymitis.

Case 10 had few gonococci in his urethra as in each of the microscopic fields of a smear of the pus only a few organisms were seen. Irrigation with 1 in 5,000 permanganate of potash was begun from the date of admission. Injections of 20 million "local" vaccine were given on April 30, 1911, and on May 3 and 11. The discharge had ceased by the latter date and only a faint haze was visible in the morning urine; 30 million were injected on May 17. Double orchitis supervened on the 19th. As the urine cleared rapidly a further dose of 30 million was tried; the reaction was slight, and a reappearance of pus from the urethra occurred. In six days there was no further pain in the testicles. On June 13 both urethra and testicles were normal, and no further evidence of gonorrhœa has occurred.

In this case vaccine treatment did not prevent the onset of complications, but that a curative influence was exercised on the course of the disease is shown by the fact that twelve days from the date of onset of orchitis the urethra was clear and the pain and swelling had gone from the testicles.

Case 11. There was acute left epididymitis present on admission to hospital; the patient had been treated for gonorrhœa some months previous to the present attack. Gonococci were very few in number. No culture was attempted. As a routine treatment Condy's fluid irrigation was employed, in a strength of 1 in 5,000. Three weekly injections, 20, 32 and 20 million respectively, of "local" stock were administered; these succeeded in curing the epididymitis, and no further trouble has been noted, nor has a relapse occurred.

A slight testicular reaction followed the first dose, but the others were not felt locally or generally.

Acute Cases.

These are divided into two groups. Nine were treated by a locally made stock vaccine and ten with a "manufacturer's" stock. Of the patients treated by the "local" stock, none had a higher dose than 50 million, the usual dose was 20 million, and the interval one week. The final dose in most of the cases was the largest, but the dose was not varied in a number of the cases, beginning at 20 million and ending with the same quantity. Reactions were avoided as far as possible and only occurred in two of the cases; 20 million appeared to be a medium initial quantity of vaccine, and the average case did not show signs of overdosage. Of course this definite statement only applies to this special vaccine, as it might be an excessive dose for a preparation made with a different technique, or with other strains of the organism. The gonococcus was isolated in pure culture from three cases of this series, and added to the stock emulsion.

The average number of days under treatment was 50·4. The shortest time was 19, and the longest 110 days, but this was an exceptional case which had a stricture in the anterior urethra, and was not in the least benefited by vaccine.

A few other cases may be quoted, first those whom immunization seemed to affect favourably, and also in whom no change could be imputed to vaccination, either for better or worse.

Case 12 was infected on April 24, 1911, and came under observation on May 7. Gonococci were few and cultivation was not successful. Irrigation with Condy's fluid 1 in 5,000 was begun on the first day. A dose of 20 million of a 10-valent vaccine was injected on May 10, 1911, and again on May 17, 1911, and was followed by rapid disappearance of both the discharge and the urinary deposit. There were no signs of gonorrhœa on May 22, 1911, and all treatment was discontinued on May 25, 1911. No relapse has occurred for nine months and no signs could be elicited on examination on March 1, 1912.

Case 13. This was his first attack. He became a hospital patient on February 26, 1911. Examination of the copious purulent discharge from the urethra showed a pure gonococcal infection to be present, very numerous organisms being found both free and in the cells. A culture on the special medium yielded a large growth; this was added to and formed part of the polyvalent vaccine. The urethra was flushed out thrice daily with permanganate solution until March 21; there was no discharge on that

date, though a haze could be seen in both the first and second parts of the morning urine. A dose of 20 million of the above-mentioned stock was given without reaction. Two days later a similar injection did not cause a negative phase, and in forty-eight hours the urine was clear. Irrigation was then stopped, and the patient left hospital on the thirty-first day. No relapse has since taken place.

Case 14. This case was a severe one. He was admitted with a urethral abscess, and a copious discharge containing a large number of gonococci ; a pure culture of the organism was obtained on the first examination of the discharge. During the first month he had irrigation with potassium permanganate in very dilute solution owing to pain in the urethra. On March 16, 1911, 20 million gonococci were injected. There was no reaction. Four days later 12 million were injected, and three days later he received a dose of 16 million cocci. At the end of the three injections he still had a discharge containing gonococci. Owing to an anterior urethral stricture sounds were passed daily until Nos. 11 to 13 sounds could be introduced through the narrowed part. Three further doses of vaccine, each of 20 million, at weekly intervals, caused the disappearance of the gonococci from the urethra, and no discharge was to be seen. Four injections of 20 million, with seven days between each of them, completed the treatment.

In this case irrigations were continued throughout the time he had vaccines. Pain was not relieved by the treatment, it disappeared gradually, and the cessation did not seem to be connected with any particular dose of vaccine. Gonococci were found during the first two and a-half months of observation. Perhaps the dosage of the organism was insufficient, as no reaction occurred during the ten vaccinations. It did not appear advisable to give a larger dose owing to the acuteness of the symptoms for a long period.

Ten cases had injections of a stock made by one of the vaccine manufacturers. The quantity most usually employed was 50 million organisms, and varied from 5 to 50 million, the latter dose was not exceeded in the acute cases.

As with the preceding cases irrigation with permanganate of potash and silver nitrate was used in addition to the vaccine. A few typical cases are here quoted.

Case 15 had a copious discharge of pus from his urethra, numerous gonococci were found microscopically, both in the pus cells and free. Five million gonococci were injected the day following his admission into hospital. No reaction resulted. On January 9, 1911, 50 million were given ; this was repeated on January

22 and 29. There was a distinct reaction after the last injection, with an increase of urethral discharge, which had previously been getting less. In a few days the condition cleared up very rapidly and no signs of the disease were present in the urethra or urine. The patient was permitted to leave hospital a week later. No relapse has since occurred. The duration of his stay in hospital was thirty-five days ; during the last week of this time he was apparently free from gonorrhœa.

In Case 16 gonococcus was the only organism present, but an attempt to grow it was a failure. Vaccination was carried out weekly. The first dose was 25 million, the second 50, the third was also 50 million, and no reaction occurred after any of these, but at the same time there was no appreciable change in the number of gonococci or in the amount of his urethral discharge. The fourth injection was reduced to 20 million ; both gonococci and discharge diminished in the course of a week. The disease became quiescent and it was thought that a cure had been brought about. However, in a week both gonococci and discharge reappeared and vaccines were resumed. Two doses, 50 million in each, were given with an interval of a fortnight. No further recurrence has taken place for a year. This case had no other treatment but the stock vaccine. He was under treatment for exactly two months. The next case was treated in a similar manner, inoculation alone being used.

Case 17. When admitted on November 12, 1910, the urine contained much mucus and many threads. Gonococci were found scantily in the threads, but cultivation of the organism was unsuccessful. Urethroscopic examination revealed a granular and swollen state of the whole urethra, as far as could be seen by means of Casper's instrument.

On November 29, 1910, he was injected with a dose of 5 million stock vaccine. There was no reaction. At weekly intervals three doses each of 50 million gonococci were given, and on December 20, 1910, neither urethral discharge nor Thompson's "two glass" test showed any evidence of gonorrhœa. A final dose of 25 million on January 2, 1911, completed the treatment. This case was in hospital for fifty-three days ; he was clear of signs in forty days. No other form of treatment was employed in this case, and no relapse has occurred in twelve months.

The other cases do not present points of any special interest. The accompanying table shows the results of this form of treatment.

	Acute Gonorrhœa.	
	No. of cases	Average No. of days in hospital.
Manufacturers' vaccine	10	48·9
Local stock vaccine	9	50·4

For at least one week of this time in hospital there were no signs of disease, as tests were carried out to prove that the gonorrhœa had ceased. By acute gonorrhœa is meant those cases which were treated from the time the men themselves noticed a discharge from the urethra. It is unusual for these men to suffer pain or scalding in the earliest stage of the disease, hence they do not come under treatment till there is a thick yellow discharge of pus from the urethra.

Although these thirty cases were in hospital more than the average time, relapses were below the average, and were quickly amenable to the treatment.

Whether dosage can be laid down is doubtful, for some men could tolerate larger doses of vaccine than others. No true anaphylactic symptoms due to the gonococcus occurred. In some cases a negative phase was observed, but this was due to an overdose.

As in other vaccines too small a dose is to be avoided for it would appear that immunity against the vaccine is the only result, *vide* Case 14.

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A French method of utilizing a taxicab chassis exists (see fig. page 456). The principle is the carriage of a Bréchot-Desprez Améline frame upon a platform which is supported on two wooden cross beams fixed to the chassis frame. It will be seen that there is an "overhang" and that the centre of gravity is thrown high, especially if the upper tier were occupied. The hinder cross bar is not longer than the width of the chassis frame and comes between the hind wheels; it would not carry a frame any wider than that now seen upon it.

A CASE OF BLACKWATER FEVER AND A SUGGESTION.

By Captain D. S. SKELTON.
Royal Army Medical Corps.

The following case presents features that seem in some respects so out of the common as to make it worth putting on record.

Captain P., Royal Engineers, aged 30, during a morning's inspection work at Strensall, noticed that he was passing red urine; he had been feeling seedy all that morning. He consulted an officer of the R.A.M.C. at the Camp, who advised him to return home at once, go to bed and send for medical assistance. This advice was followed. At 2 p.m., that was two hours after he had noticed the "red" urine, he passed a small amount of "porter" coloured urine. The temperature at this time was 100·5°. There was no vomiting and no obvious jaundice. Captain Patch and Lieutenant Levack, R.A.M.C., both saw the case. The temperature was normal at 5 p.m. and at 9 p.m. The urine at 5 p.m. was "porter" coloured, about 70 c.c. being passed. At 7 p.m. it was much clearer. I took over the case next morning. The temperature was normal, the urine quite clear, and the patient felt much better. Specimens of the urine passed the previous afternoon and evening had been kept for me to see. Without doubt they were typical "blackwater" specimens.

The history of the case is interesting. Captain P. went out to Sierra Leone in 1903, and completed his first tour of service. After the usual period of leave he returned to the Coast, from which he was eventually invalided in August, 1906, after a very severe attack of blackwater fever. He gives a history of having had a good deal of malaria during his West Coast tour, in fact as many as half a dozen well-marked attacks. Four months ago, that is five years and four months after leaving the Coast, he mentions having suffered from attacks of general seediness, which a civil medical practitioner diagnosed as influenza, but they were only slight, and were treated with a mixture. Except for this mixture, not all of which was consumed, no quinine in any form has been taken for 5½ years. The patient thinks that during the past six months his urine has been getting thicker. Specimens of the "porter" coloured urine

passed at the time of the attack were brought to the laboratory, and Captain Hayes, R.A.M.C., kindly assisted me in their examination. Examination of the urine under the microscope showed no red blood corpuscles, but granular casts were numerous.

The "blackwater" specimens contained albumin (a half). Several blood films were taken, one at the time of the paroxysm, the others next morning, but though Captain Hayes and I both searched for nearly two hours no parasites were found. A differential leucocyte count gave a mononuclear percentage of 7·5. On the third day the urine was quite clear and contained no albumin.

The treatment consisted in the first instance of the administration of a mixture containing liq. hydrag. perchlor. and sodæ bicarb. and later of a diuretic mixture with smart calomel purging. No quinine was given.

Second Attack.—On Monday, May 8, having just been taken off the sick list, he again began to feel seedy. At about 4 p.m. he started shivering. He passed water at 5 p.m. It was clear. At 7 p.m. he passed about 70 c.c. of "porter" coloured urine. The temperature at 9.20 p.m. was 101.

Next morning he looked very ill. There was slight jaundice, and he had that almost characteristic ashy grey colour which is associated with severe paludism. The urine cleared in the course of the day. The temperature was normal. I gave him quinine sulphate, 5 gr. doses to be taken three times a day. He continued to take this mixture till Sunday the 14th. On the 15th I expected that he might have another attack, but he did not. I assume that the quinine had done its work. There was no attack on the 22nd or on the 24th. When I last saw him he was quite well.

A medical board was held on him and long leave was recommended in order that he might go to the South.

He was advised to take ten grains of quinine each Monday and Tuesday for the next three months.

Remarks.—It is by no means rare for cases of blackwater fever to occur in England, when the patient has recently returned from tropical Africa. I myself have met with three such cases. They were all mild in type. But the case I have described above is remarkable, in that no less than 5 years and 8 months have elapsed since the patient left the West Coast. Another point is that, except for a few doses of a mixture for influenza (!) the patient had taken no quinine at all for all this time. In any case, absolutely *no* quinine had been given for four months. This surely rules out quinine as being an invariable etiological factor in the production of "blackwater," a contention that I, personally, have always held.

Supporters of the quinine school are, of course, entitled to say that the case of Captain P. was not one of "blackwater" at all. Against that I can only urge a considerable experience of "blackwater" and the want of an alternative diagnosis. The only other possible diagnoses are acute neph-

ritis and paroxysmal hæmoglobinuria. The former is ruled out, however, by the absence of red blood cells in the urine or any of the other classical signs and symptoms. The second is merely a term—a symptom with some definable cause, such as either malaria, poisonous drugs or quinine. It is not a disease *per se*. To my mind the case of Captain P. offers no doubt in the matter of a diagnosis. It was a typical mild attack of "blackwater" such as paludics not uncommonly get on returning to England. The failure to find any parasite in the blood is a common event, as after the paroxysm the parasite generally disappears from the peripheral circulation.

My idea as to the origin of such attacks of malaria after a prolonged period of immunity is that the parasite suddenly develops by parthenogenesis. No doubt the number of parasites in the host is remarkably small, hence the mildness of the attacks. The intense "blackwater" of malarial Africa is associated with all the symptoms of a virulent toxæmia, vomiting, coma, and suppression of urine. That the coma is not due to the suppression is clear, in that it frequently comes on early, in fact almost within a few hours of the onset of the attack.

Our conceptions as to the real cause of blackwater fever have not progressed much during the past five years.

Bentley and Christophers have attempted to explain the mechanism of the production of the blackwater. They have experimentally produced a lysæmia, which, they say, is brought about by an auto-hæmolysin. But then again other workers deny that an auto-hæmolysin is ever produced.

Barrett and Yorke are convinced that quinine produces the hæmolysis. They maintain that blackwater is not due to a hæmolysin. The same workers show that the suppression of urine, which is such a fatal complication, is due entirely to mechanical causes, a position I maintained in 1908. They find granular and epithelial casts in the urine of a rabbit some 12½ hours after the intravenous injection of dissolved hæmoglobin, whilst granular casts are commonly found in "porter" coloured urine.

Simpson has described a case studied before and during the malarial paroxysm. The parasites disappeared with the onset of the hæmoglobinuria. This, however, is the common experience of workers on the West Coast, that is to say the parasites disappear from the peripheral circulation.

In the case of Captain P. we have a malarial infection, the recrudescence of which can only be due to the parasite re-developing by parthenogenesis, and the same must be true as a general rule in all cases where the disease has been quiescent for any prolonged period. Have we then *here* a factor in the production of "blackwater" in general, which will help to explain in any way the elusive nature of this disease? When the parasite develops by parthenogenesis are the products of metabolism more intensely toxic than when development is by sporogony and schizogony? Much work has followed that of Ehrlich and Sachs on hæmolysins, and

the results have been applied to the production of hæmoglobinuria. But no one has explained why we get "blackwater" only in certain parts of Africa, India and the East Indies. No one has explained why we do not get "blackwater" whenever we get malignant tertian malaria.

As to the cause of the "blackwater" fever, might we not be dealing with certain "strains" of the *Plasmodium malariæ ?* Is it not possible to conceive of certain "strains" as being especially resistant to the immune bodies that are produced in the hosts? And going still further, can we not produce a strain which becomes a "super-parasite," and is resistant to quinine? Owing to unfavourable surroundings and conditions, such as the presence of antibodies in the host, &c., development may be hindered for prolonged periods, until finally parthenogenesis may occur if the species is to be perpetuated. Can it not be conceived that the large parthenogenetic forms of the *P. malariæ*, containing as they do a large amount of nuclear matter and pigment, are so intensely toxic in their products as to give rise to the condition of "blackwater"? This theory of compulsory parthenogenesis in its relation to the production of "blackwater" fever would fit in with the sudden onset and the sporadic nature of the disease, and it would also explain why paludics who have been taking quinine suddenly get "blackwater." Under the influence of quinine,[1] it can be conceived that the parasite will assume its most resistant form in its struggle for survival, an analogy to the granular stage in the life history of spirochætidæ, so that, in other words, quinine can either cure the disease or be the innocent cause of inducing the formation of the highly resistant and intensely toxic parthenogenetic form of Plasmodium.

Finally the moral of this case of Captain P. appears to be that when once a West Coast paludic has had "blackwater" we cannot promise that he will not get it again, even after an interval of years. This fact is to be commended to those who sit at home imagining that men may nowadays go out with impunity to the Coast for their health or for shooting.

It is surely an argument against any curtailment of West Coast leave, pay or privilege.

I am indebted to Lieut.-Col. N. Ferguson, C.M.G., for permission to publish this case.

[1] The administration of quinine is said to induce crescent formation.

THE TREATMENT OF GONORRHŒA AND SOME OF ITS COMPLICATIONS.

By Major L. W. HARRISON and Lieutenant C. H. H. HAROLD.
Royal Army Medical Corps.

A study of the literature on gonorrhœa reveals an astonishing number of methods of treating this disease. Many of them are credited with the power of curing it in anything from a few days to a week or so, and some of these must have been tried by everyone who has treated gonorrhœa. Yet the average duration of stay in Army hospitals for gonorrhœa is thirty-three days; about 15 per cent of admissions for gonorrhœa are for relapses; very large numbers of women are chronic invalids and quite one half of gynæcological operations are performed on account of gonococcal infections generally contracted through marriage with men who believed themselves cured, while a very large amount of blindness from infancy is due to the same cause.

Clearly the cure of gonorrhœa is not so sure and easy as the authors of these numerous remedies would have us believe, and their claims must have been based on tests of cure which were insufficient.

Some time ago we determined to ascertain how far clinical evidence could be relied on as a test of cure in gonorrhœa. Accordingly we examined for the presence of gonococci fifteen patients who had been suffering from gonorrhœa, and, under irrigation with weak permanganate of potash solution, had apparently recovered Some days after suspending treatment and administering beer no discharge could be expressed from the urethra by massaging it from the bulb forwards, and the urine was clear and free from threads. The examination was conducted in the following manner, which we have adopted in the case of every patient we have since treated for gonorrhœa before discharging him from hospital.

The urethra was first washed out with physiological salt solution, some of which was finally injected into the bladder. The prostate was then thoroughly massaged for a few minutes and a specimen of any secretion which was expressed from the meatus in this way was examined microscopically for gonococci. At the same time the patient was made to pass into a urine glass the salt solution which had been injected into his bladder. If no gonococci were found in the secretion expressed directly from the meatus the salt solution

was centrifugalized and some of the deposit examined in the same way. The specimens were stained by Gram's method.

We may mention here that on massaging the prostate for the first time a large amount of prostatic secretion often appears and obscures the details in the microscopic specimen, making the detection of gonococci difficult. If, however, the prostate be massaged again on the following day the amount of glairy prostatic secretion is less and the specimen easier to examine.

By this method we found that every one of the fifteen cases were harbouring gonococci, and most of them in large numbers. Subsequently we retained these patients in hospital for a further period and attempted to free them from gonococci, but eventually had to discharge them, clinically cured of gonorrhœa, but really harbouring gonococci and consequently candidates for relapse. None of them showed any gonorrhœal secretion during the period of this later treatment, and, needless to say, all of them, considering themselves cured, thoroughly disagreed with our line of action.

It was quite clear to us after this preliminary examination that clinical evidence, *i.e.*, absence of discharge from the urethra and of threads from the urine, is no criterion of cure. Indeed, we have since found that failure to find gonococci after the test we have described is not a certain indication of their absence, because on several occasions we have failed to find them twice in succession and succeeded at the third attempt on the same case. We think, however, that this test is a more certain means of ascertaining the value of any particular line of treatment than clinical evidence or microscopical examination of secretion obtained only from the anterior urethra, and as it is comparatively easy to carry out we recommend it to anyone who is anxious to test a new method of treating gonorrhœa.

On the next favourable opportunity we commenced an investigation to discover, if possible, a method of treating gonorrhœa which would shorten the stay in hospital, prevent complications, and, by freeing the urethra more completely from gonococci would reduce the number of relapses. Our efforts have not been crowned with very brilliant success, but we have ascertained a few facts which may be useful to those who have to treat this disease.

Previously one of us had obtained evidence that the use of gonococcal vaccine has some effect in preventing the complications of gonorrhœa. Briefly, in two parallel series of cases which were treated identically by the same medical officer except that one received injections of vaccine, the incidence of

epididymitis in these was less than half of that which occurred in the series which received no vaccine.

We had also reason to believe that the use of atropine has some effect in preventing epididymitis. The use of atropine with this object was suggested by Schindler (*Berl. klin. Woch.*, September 3, 1909, p. 1691), who found by experiments on animals that when the posterior urethra was inflamed and was irritated, either directly or through massage of the prostate, a reverse contraction of the involuntary muscle fibres of the vas occurred. He believes that this is the mechanism by which gonococci lying in the posterior urethra are carried to the epididymis, and that it explains how it is that so many cases develop epididymitis a few hours after a metal instrument has been passed along the inflamed urethra. Confirming the findings of Bumm and Leopold, Schindler also found that when the hypogastric plexus was paralysed by administering atropine, either hypodermically or in the form of suppositories, this reverse contraction did not take place. The following case appears to support Schindler's belief. A patient in the Military Hospital, Rochester Row, had suffered from six attacks of epididymitis since admission. Each attack had immediately followed any attempt to massage his prostate and seminal vesicles or to administer local treatment to his urethra. His prostate was considerably enlarged and both seminal vesicles were tightly distended, while all these parts were very tender to palpation. He had also suffered from repeated attacks of arthritis which had spared very few of his joints. It seemed clear that unless the focus of infection in his prostate, &c., were dealt with he would continue to suffer from attacks of arthritis. But massage of the prostate and, in fact, any local treatment was always followed by epididymitis, and for this reason the patient himself dreaded any local interference. In the hope of breaking this vicious circle, an atropine suppository was administered in the evening and another the following morning. An hour after the second suppository the prostate and seminal vesicles were massaged as thoroughly as the patient could tolerate. No attack of epididymitis followed this time, and the massage was continued, at first on alternate days and then daily, while the urethra was treated with daily injections of protargol. The prostate and seminal vesicles gradually improved and shrunk to their normal size, no further attacks of arthritis occurred, and the patient was eventually returned to duty, at which he has continued for more than a year.

Impressed by this case we have since administered atropine

suppositories ($\frac{1}{75}$ gr. in each) twice daily to each of our gonorrhœa cases and attribute to this some reduction in the incidence of epididymitis, as well as the marked freedom of our patients from painful erections and chordee.

As is well known, opinions on the treatment of gonorrhœa are broadly divided into two schools : (1) Those who consider it essential to treat the urethra locally, while observing the rules for general treatment, and (2) those who consider that local treatment is of no value, or even harmful, and rely entirely on general treatment with internal medication.

With regard to local treatment, our experience had led us to believe that better results could be expected in the acute stage from irrigation of the urethra with fairly large quantities of some bland liquid like weak permanganate of potash solution or physiological salt solution than by applying to it stronger bactericidal preparations such as protargol or silver nitrate. In fact, we had found that when protargol ($\frac{1}{4}$ or $\frac{1}{2}$ per cent) was used in the acute stage the urethra was kept in an irritable condition for a longer time, while the discharge continued to a later date and epididymitis seemed to occur more frequently.

We concluded from this that if local treatment were of any value in the acute stage it must act mechanically by removing gonococci and their toxins, as well as diluting the latter.

Regarding internal medication, we had not witnessed such brilliant results from the use of sandal-wood and copaiba preparations as to convince us that we could expect any marked benefit from their use. In fact, the only specific internal remedy which we believed might affect gonorrhœa more favourably was gonococcal vaccine.

In order to determine in a general way the comparative values of (*a*) purely local treatment, (*b*) purely general treatment, with the help of gonococcal vaccine, and (*c*) local and general treatment with the help of vaccine, we divided our freshly admitted cases of uncomplicated gonorrhœa into three series. The first was treated with large irrigations of physiological salt solution three times a day, using half a gallon of solution for each irrigation. In the second no local treatment was administered, but a small dose of gonococcal vaccine was injected twice a week, while the third series had irrigations like the first and vaccine like the second. All the patients were ordered rest and diet as is customary with gonorrhœa, and each had an atropine suppository twice daily.

In the course of a few weeks we found that the progress of

the third series (local treatment plus vaccine) was the best. The disease pursued a milder course, and the discharge ceased in a shorter time than in either of the other two, while quite a large proportion showed no gonococci when the test was applied after the discharge had ceased for a few days. Those who were treated with vaccine only were making very slow progress when, in fairness to them, the experiment was concluded and local treatment commenced. In those who received irrigations only the disease pursued much the same course as under irrigations with permanganate solution, but, if anything, they were rather longer in clearing up. It was easy to find gonococci in both these series some days after the discharge had ceased.

Concluding from this preliminary investigation that the best results were to be expected from vaccine combined with local treatment, we then tried to improve the local treatment.

Interpreting the relations between gonococci and the urethral tissues in the light of pathology and symptoms it appears as if invaders and invaded waged a fierce battle at first, but that the tissues eventually came to tolerate the micro-organisms under mild protest; a protest which is insufficient to remove them just when a little further effort might succeed. For this reason we judged it well after the inflammation had considerably subsided to try the effect of a mildly stimulating and bactericidal application to the urethra, and modified the local treatment thus.

After irrigating with physiological salt solution throughout the acute stage till the discharge had become considerably less, the urethra was treated with the following solution:—

℞ Cupri. sulph .. .. ¼ gr.
Zinc. sulph .. .. ½ „
Aq. .. .. ad ℥i

a few ounces being injected thrice daily after the usual irrigation. The change effected a great improvement. In some cases the discharge ceased in a week or ten days after admission to hospital, and in the majority in about seventeen days. The urethral secretion was purulent till it disappeared, and a few days later the urine was clear and free from threads. In this respect there was not a very marked improvement on treatment with irrigations of permanganate of potash solution, but examination of any secretion which could be expressed by massaging the prostate and anterior urethra showed that in a large proportion of cases treated in the way we have described gonococci could not be found, and these

were certainly never so numerous as in any of the patients treated by the other methods we have mentioned.

Lately we have substituted weak permanganate of potash solution for the saline as an irrigating fluid and find that the discharge becomes less in a rather shorter time, so that the astringent solution can be added to the treatment at an earlier date. Possibly, as Major Pollock has suggested, the permanganate solution neutralizes the gonotoxin to some extent so that this effect is added to that of removing mechanically the micro-organisms and their toxins.

We have also tried the effect of substituting protargol solution ($\frac{1}{4}$ per cent) for the zinc and copper, but have not found that our cases progressed better under its use. Schindler has lately recommended that in cases which do not clear up quickly, and the disease appears to be passing into the chronic stage, a protargol jelly should be administered. The prescription is as follows:—

℞	Agar jelly (2·5 per cent)	40
	Dissolve with gentle heat and add distilled water ..	160
	When cold rub up with protargol	1

We have tried this jelly on a number of cases and find that it is useful if not commenced too early. The agar basis has the effect of maintaining the protargol in contact with the urethral wall for a longer time than is possible with a watery solution. If commenced too early, however, before the acute stage has well subsided, it seems to keep the urethra in an irritable condition and delays recovery. Possibly we have made our patients retain it in the urethra too long, and it would be better to expel it by urination or other means after ten minutes or a quarter of an hour. In the majority of cases we have found that the injection of protargol jelly has produced a thick discharge which was apparent the following morning, the secretion containing numerous polynuclear cells, but few or no gonococci. In some cases after a thick discharge had followed the protargol jelly in this way for a few days it ceased, and on applying the test no gonococci could be found. As far as we can judge at present, therefore, we think that protargol jelly will prove a useful adjunct to other local treatment, but should not be commenced too early, nor should the jelly be allowed to remain in the urethra too long.

To complete the account of our investigation into the local treatment of gonorrhœa we may mention that we have tried ionic medication of the urethra in a few cases in which gonococci

persisted after other symptoms had cleared up. The treatment was applied in the following way. After injecting some sulphate of zinc solution ($\frac{1}{4}$ per cent) into the bladder a specially made gum elastic catheter provided with a number of small perforations in its sides and containing a pure zinc stylet round which the mouth of the catheter was fastened to prevent any solution escaping, was passed. The zinc electrode was connected to the positive pole of a constant current battery, the negative pole being connected with an electrode applied to some other part of the patient's body. A current of 5 milliamperes was passed for five minutes. The effect in each case was to cause a purulent urethral discharge, which ceased in a few days, and examination then showed a reduction in the number of gonococci, while in some they could not be found. We have not tried this method of treatment sufficiently, however, to report definitely on its merits, but would not use it in the acute stages of gonorrhœa.

To summarize the results of our investigation into the local treatment of gonorrhœa we have obtained the quickest apparent recoveries from the following procedure :

(1) Irrigation of the urethra three times a day with large quantities of weak permanganate of potash solution (1 in 3,000 to 1 in 4,000) till the discharge has considerably diminished. Then

(2) Injection of zinc and copper solution after each irrigation till the discharge has practically ceased and, finally,

(3) Injections of protargol jelly in place of the zinc and copper after massaging the prostate and irrigating the urethra.

(4) Atropine suppositories ($\frac{1}{75}$ gr. in each) twice daily.

Confirming the observations of others, we think it important for the prevention of epididymitis that the following precautions should be observed :—

(*a*) The irrigator should not be more than five feet above the urethra; (*b*) the temperature of the irrigating or injection fluid should not be greatly above or below that of the body; (*c*) strongly irritating fluids should not be injected; and (*d*) the patient should rest in bed for the first week and duty should be light till the discharge has ceased for a few weeks. Pack-drill immediately on leaving hospital seems to be a very potent factor in producing epididymitis. It is worth while taking considerable pains to prevent epididymitis, since these cases are by far the most intractable gonococcus carriers and the most prone to relapse, so that they spend a considerably longer time in hospital than uncomplicated cases of gonorrhœa.

Regarding the vaccine therapy of gonococcal infections, opinions are divided. Probably most workers agree that it is generally useful in the complications of gonorrhœa, but few consider it of any value in gonorrhœal urethritis. Our experience leads us to believe that gonococcal vaccine may usefully be employed not only in the treatment of complications but in ordinary cases of gonorrhœa. Considering the beneficial effect of vaccine on complications of gonorrhœa it is reasonable to suppose that when the gonococcus passes into the blood-stream and invades other parts of the body, generally speaking, it is not in such a favourable medium as on the urethral mucous membrane. If, therefore, the anti-gonococcal substances in the blood can be increased gonococci will be destroyed as soon as they come under their influence, and complications will be nipped in the bud. Further, in a patient whose resistance to the gonococcus had been raised the micro-organism could not penetrate so deeply below the surface of the urethral mucous membrane as in other cases, since in doing so it would come within the sphere of influence of the blood. In other words, the inflammation should be a more superficial one.

We have mentioned that in a series of cases in which we administered gonococcal vaccine with the object of preventing complications the incidence of these was less than half the number which occurred in a similar series treated without vaccine. Incidentally it was also noted that the cases treated with vaccine did not suffer from periurethral thickening to the same extent as the others.

We think that it is useful to continue vaccine treatment after the patient has left hospital, with a view to preventing relapses. Even failure to find gonococci after three tests such as we described in the beginning of this paper is not a certain indication of complete absence. For this reason we have considered that quite possibly all the cases of gonorrhœa we have discharged from hospital were gonococcus carriers, and have made as many as possible of them attend as out-patients for a few weeks afterwards. Out of 115 patients discharged from hospital 91 attended for vaccine and 2 relapsed, both being men who had suffered from epididymitis, and one of these a patient who was specially discharged for private reasons before the inflammation had properly subsided; out of 24 who could not attend 6 relapsed, three being old epididymitis cases. It seems, therefore, as if continued treatment with gonococcal vaccine had been beneficial in preventing relapses.

The vaccine we have used was prepared at Rochester Row from

cultures isolated from eight or ten different sources. It was killed by the addition of 0·5 per cent carbolic acid. As regards dosage, we have been guided by clinical symptoms. With one batch of vaccine we were able to inject 25 millions without producing any marked increase of the discharge or general disturbance, but with later batches we have found it advisable to commence in acute cases of gonorrhœa or of arthritis with a dose of 2·5 millions, increasing this to 5 millions three or four days later if no marked disturbance followed. We do not exceed 5 millions twice weekly till the gonorrhœal discharge has ceased. After this it is increased to 12·5 to 25 millions, and in more chronic cases may usefully be increased to 50 or 100 millions. When the larger doses are given the vaccine is administered once a week.

Irons and others have suggested that gonococcal vaccine may have some diagnostic value, acting in a manner analogous to tuberculin by producing a local and general reaction in the presence of a gonococcal infection. This is supported by the increase of gonorrhœal discharge which numerous observers have found after administering the specific vaccine. We have frequently noticed this increase of discharge and have reason to believe that gonococcal vaccine produces a local and general reaction in the presence of other gonococcal lesions. Patients suffering from complications frequently have some slight temporary increase of pain a few hours after the injection of vaccine, and in a few cases where it was doubtful whether a certain train of symptoms depended on the gonococcus or not, the injection of a dose of vaccine enabled us to settle the point. In illustration of this, a patient who was suffering from rheumatism of the feet had not had a gonorrhœal discharge for eighteen months. Examination by the test we have described failed to reveal any gonococci in his urethra on three occasions. A dose of gonococcal vaccine (25 millions) was injected and was followed by a marked increase of pain in the feet and reddening round the joints, as well as a slight urethral discharge in which gonococci were found. Another patient who had suffered intermittently from gleet for some years had been examined by a number of experts who could not give a definite opinion as to its gonococcal nature. Our first examination was negative, but on the day after injecting a dose of gonococcal vaccine the diagnosis was established by finding gonococci in the urethral discharge which appeared.

We can confirm the observations of those who have found vaccine of value in gonococcal metastases. We have obtained excellent results from its use in cases of arthritis, sclerotitis, epididy-

mitis, periostitis, and teno-synovitis. In some cases, however, it has proved disappointing, and these have been patients who were suffering from very acute arthritis attacking several joints in succession and accompanied by severe general symptoms. Possibly our dosage was faulty in these cases, erring on the side of over-dose, and we have certainly found that these very acute cases ran a more favourable course under injections of 2·5 millions of gonococcal vaccine or less twice weekly than under larger doses.

In the treatment of the more severe grades of gonococcal arthritis we have been impressed by the benefit which resulted from keeping the joints at absolute rest. In two cases, one of arthritis of the hip-joint, the other with considerable effusion into the knee, no improvement occurred till mild extension was applied to the joint. After this progress towards recovery was very rapid. In addition to loss of sleep lowering the patient's resistance, it is very probable that lack of complete rest to the joint results in heavy overdosing with gonotoxin by auto-inoculation.

Ionic medication (using chlorine ions) has proved very useful in removing the adhesions of partly anchylosed joints, and its effect on joints which were distended with fluid has been very striking. Frequently we have seen a knee-joint which was tightly distended with fluid reduced almost to normal dimensions the day after applying ionic medication to it. It is necessary, however, to persevere with the treatment regularly for some weeks to obtain a cure. Otherwise the joint soon relapses to its former condition. We found little or no benefit from ionic treatment of a flat-foot due to gonococcal infection, but this is hardly surprising considering the anatomical deformity which had occurred.

In conclusion, we would say that there is at present no royal road to success in the treatment of gonorrhœa, but that by attention to detail much can be done to shorten the stay in hospital and to prevent complications and relapses. All of these are matters of importance considering the very large numbers of patients who are admitted yearly to Army hospitals for gonococcal infections.

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Original Communications.

PRELIMINARY NOTE ON IMMUNIZATION AGAINST *B. PARATYPHOSUS* A.

BY MAJOR S. L. CUMMINS AND MAJOR C. C. CUMMING.
Royal Army Medical Corps.

IN the JOURNAL OF THE ROYAL ARMY MEDICAL CORPS for August, 1912, Colonel R. H. Firth,[1] writing on the subject of the prevalence of enteric and paratyphoid fevers in India, makes the following statement:—

"The disturbing factor is the prevalence of paratyphoid fever, against which disease anti-enteric inoculation appears to have little influence. This view is not new, but emphasizes the plea for a bi-valent emulsion with which inoculation must be carried out against both diseases."

Some time ago, at the request of Lieutenant-Colonel Sir William Leishman, we initiated experiments with a bi-valent emulsion of *B. typhosus* and *B. paratyphosus* A, using groups of rabbits on the same lines as those employed by Sir William Leishman and his co-workers in their work on antityphoid vaccine.

These experiments are still going on, and it is not proposed, in this paper, to anticipate in any way the final conclusions of the research. The work which we now publish is only brought forward

[1] "Recent Facts as to Enteric Inoculation and the Incidence of Enteric and Paratyphoid Fevers in India." Colonel R. H. Firth.

because it appears to us to throw an interesting side-light on some of the clinical findings in paratyphoid A fever. It may also be taken to show that the question of prophylaxis against this disease is more complex than might at first sight appear to be the case, and may perhaps emphasize the necessity of a thorough experimental basis before an anti-paratyphoid A vaccine is finally recommended for the use of troops.

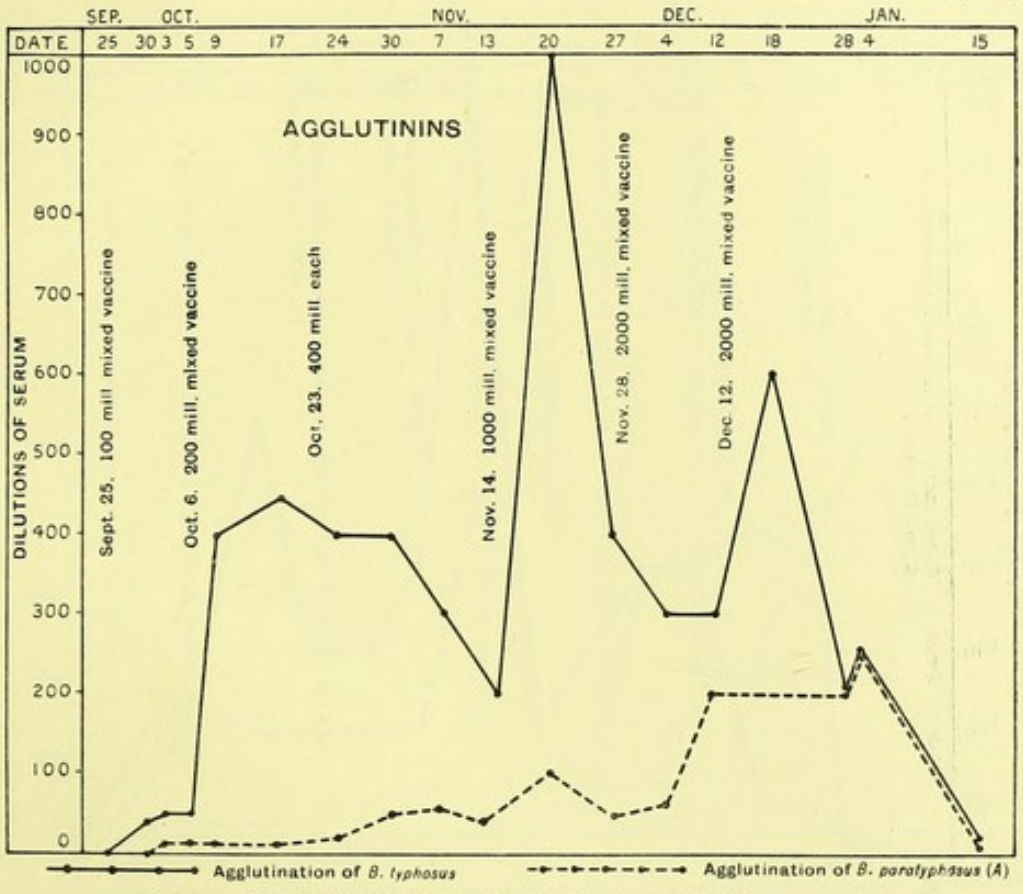
In our first experiment with a bi-valent emulsion we were surprised to find that, although satisfactory production of immune substances for *B. typhosus* followed the injections, the serum of our rabbit-groups showed only very slight response for *B. paratyphosus* A. In the case of the latter organism we failed to obtain agglutination in higher dilution than 1 in 60, and our experiments failed to elicit evidence of opsonins or bactericidins. Although Besson[1] has mentioned the impossibility of producing immunity to *B. paratyphosus* A in rabbits, we had evidence that his statement requires revision, as we had, in the laboratory, a rabbit serum capable of agglutinating this organism in a dilution of 1 in 2,000. This serum, kindly given us by Dr. Leadingham, of the Lister Institute, afforded a proof that rabbits *can* be immunized against *B. paratyphosus* A. It seemed probable that our experiment had failed through the use of insufficient dosage. Our rabbits had received a first dose of 1 c.c. and a second dose of 2 c.c. of a mixed vaccine containing 25 millions of each organism per c.c., or, in other words, a total of 75 millions of each germ, the weight-for-weight equivalent of the 1,500 million typhoid bacilli used in anti-enteric inoculation in man. Dr. Leadingham, to whom we applied for information, was good enough to look up the records of his serum, and told us that the rabbit producing it had received a series of six injections, first with killed, then with living cultures of *B. paratyphosus* A, amounting to a total of 3 c.c. of a saline emulsion of an agar slope. Various "counts" have led us to regard an emulsion of one agar slope of *B. typhosus* in 10 c.c. of saline as containing roughly 2,000 million bacilli per cubic centimetre and the same estimate will not be far out for *B. paratyphosus* A, which grows on agar with much the same facility as *B. typhosus*. It is probable that Dr. Leadingham's rabbit received something like 6,000 million bacilli in all, as compared to our 75 million, which would adequately explain our failure. But doses of equivalent amount for man would probably

[1] "Technique Microbiologique," 4th Edition. A. Besson. Paris, 1908.

cause a very severe reaction and are hardly likely to be used in practice. *The point of real importance was that, given in equal doses,* B. paratyphosus A *appeared to be a much less efficient antigen than* B. typhosus.

In September, 1911, we started a fresh series of experiments designed to ascertain what dose of *B. paratyphosus* A would produce agglutinins to a degree comparable with the agglutinin production following the usual prophylactic dose of *B. typhosus*,

CHART I.



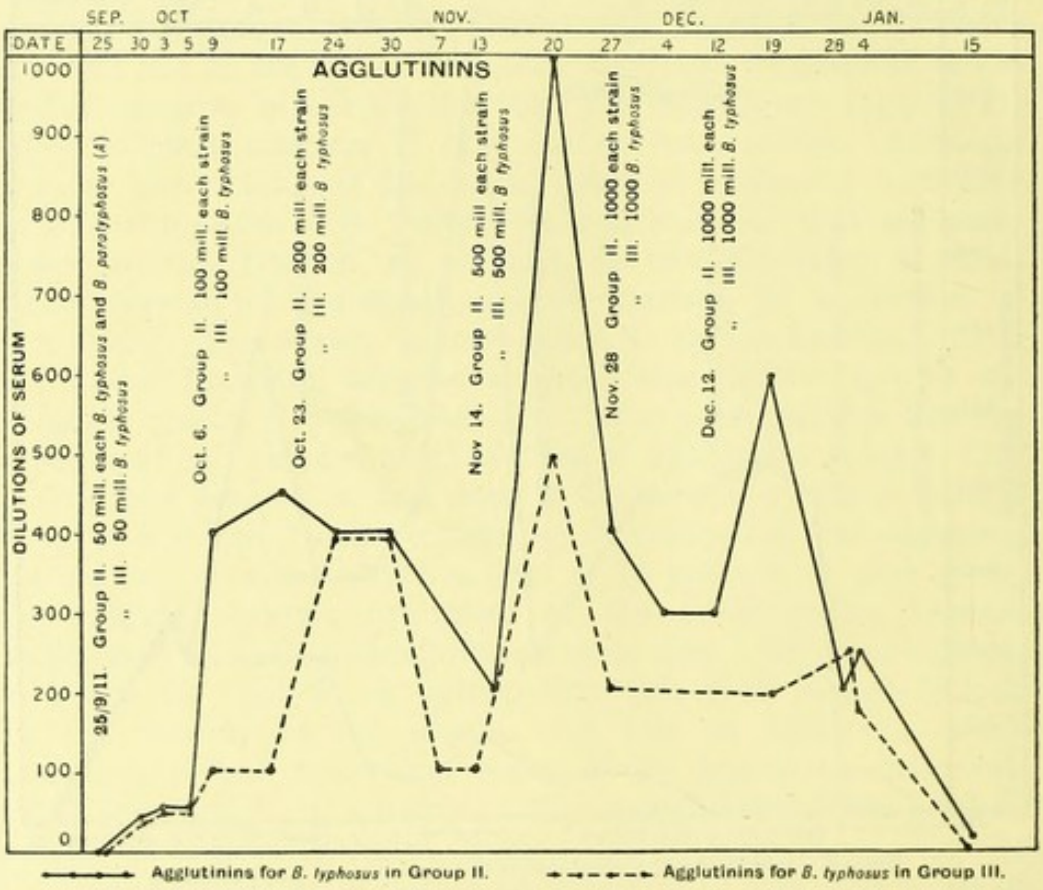
Group II. Inoculated with a mixed vaccine of *B. typhosus* and of *B. paratyphosus* (A)

The chart shows how the Agglutinins for *B. typhosus* exceeded those for *B. paratyphosus* (A)

or, rather, its equivalent for rabbits. For this purpose we used three, and finally four, groups of rabbits, three animals to each group, the weights of the different groups being equalized as far as possible. Group I was kept as a "control" and received no vaccine. Group II received a series of increasing doses of a bi-valent emulsion of *B. typhosus* and *B. paratyphosus* A. Group III was given similar doses of *B. typhosus* alone, while Group IV was treated with two inoculations amounting to 1,500

millions of *B. paratyphosus* A. All the vaccines had been killed by heat (53 ° C.), and subsequent addition of lysol. It may be mentioned here that the first experiment had contained a fallacy, inasmuch as the paratyphoid A vaccine used had been heated to 60° C. for half an hour, while the typhoid vaccine had been killed as usual at 53° C., so that the two were not strictly comparable. Chart I, however, shows that the agglutinins for paratyphoid A were greatly below those for typhoid in the second experiment as in the first.

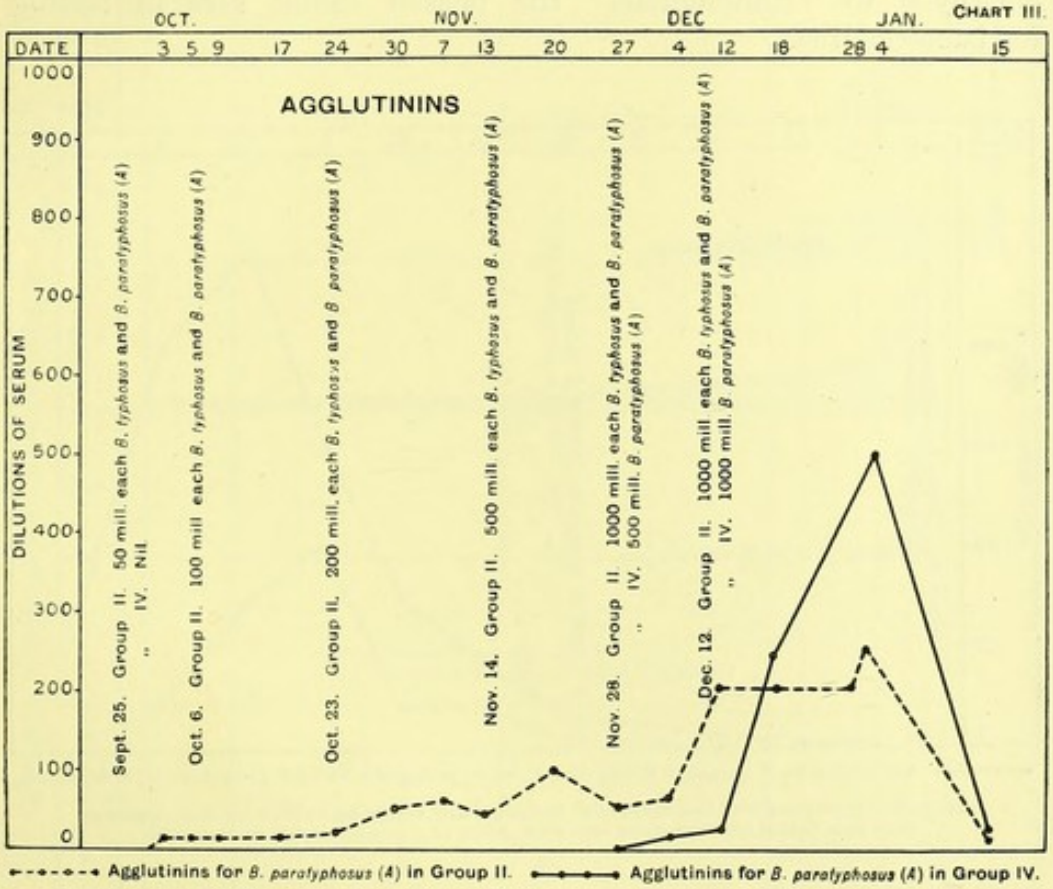
CHART II.



Group II., immunized with a mixed vaccine of *B. typhosus* and *B. paratyphosus* (A), showed higher agglutinins for *B. typhosus* than did Group III., immunized with equal doses of *B. typhosus* alone

A point of great interest emerges in Chart II, where the typhoid agglutinins produced in Group II and Group III are compared. It is here seen that the typhoid agglutinins were consistently higher in the group of rabbits immunized with a mixed vaccine than in the group immunized with *B. typhosus* alone, although the doses of this organism were the same in both. Further, in Chart III, where the paratyphoid A agglutinins in Group II and

Group IV are compared, it is seen that these were decidedly *less* after use of the mixed emulsion than after the two doses of paratyphoid A vaccine alone. This latter fact may perhaps be due to a diminished response on the part of the tissues after a long series of inoculations, as it is seen in Group III that the two last inoculations of 1,000 millions each led to but a very small response, in fact, failed to check the gradual drop of immunity. But, on the other hand, the better production of agglutinins by

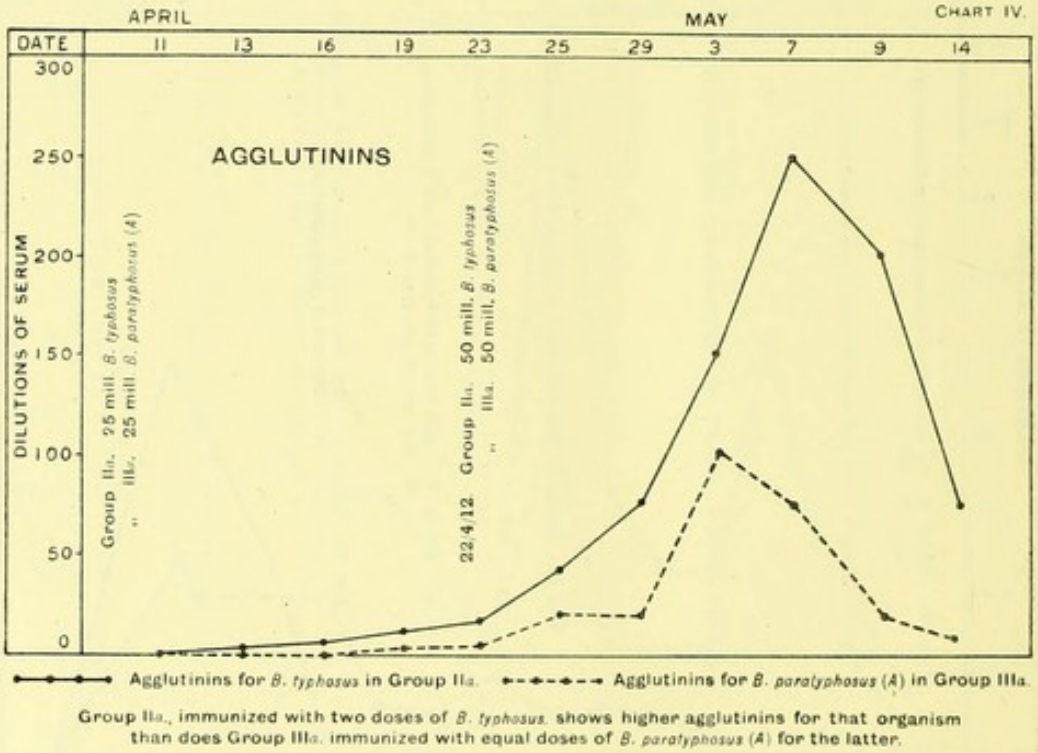


Group IV., which received two doses—one of 500 mill., the other of 1000 mill. *B. paratyphosus* (A)—showed higher agglutinins for that organism than Group II. immunized with a series of doses of a mixed vaccine of *B. paratyphosus* (A) and *B. typhosus*

an unmixed paratyphoid A vaccine as compared to that by a bi-valent emulsion may be a phenomenon complementary to that exhibited in Chart III for *B. typhosus*.

A third observation was instituted in April, 1912. On this occasion separate groups of rabbits were employed for each organism and the results compared. As before, Group Ia was retained as a control and given no treatment. Group IIa received 25 millions *B. typhosus* on April 11 and 50 millions on April 22.

Group IIIa received similar doses of *B. paratyphosus* A on the same dates. The serum was invariably collected twenty hours after the blood had been withdrawn and was heated to 58° C. for twenty minutes before testing. This heating was carried out to eliminate "complement," as this substance is difficult to estimate by quantitative tests, probably varies in the same serum at different times, and thus introduces a fallacy into examinations of sera for immune substances. Known quantities of guinea-pig serum were employed to "complement" the heated rabbit sera in testing for bactericidins.

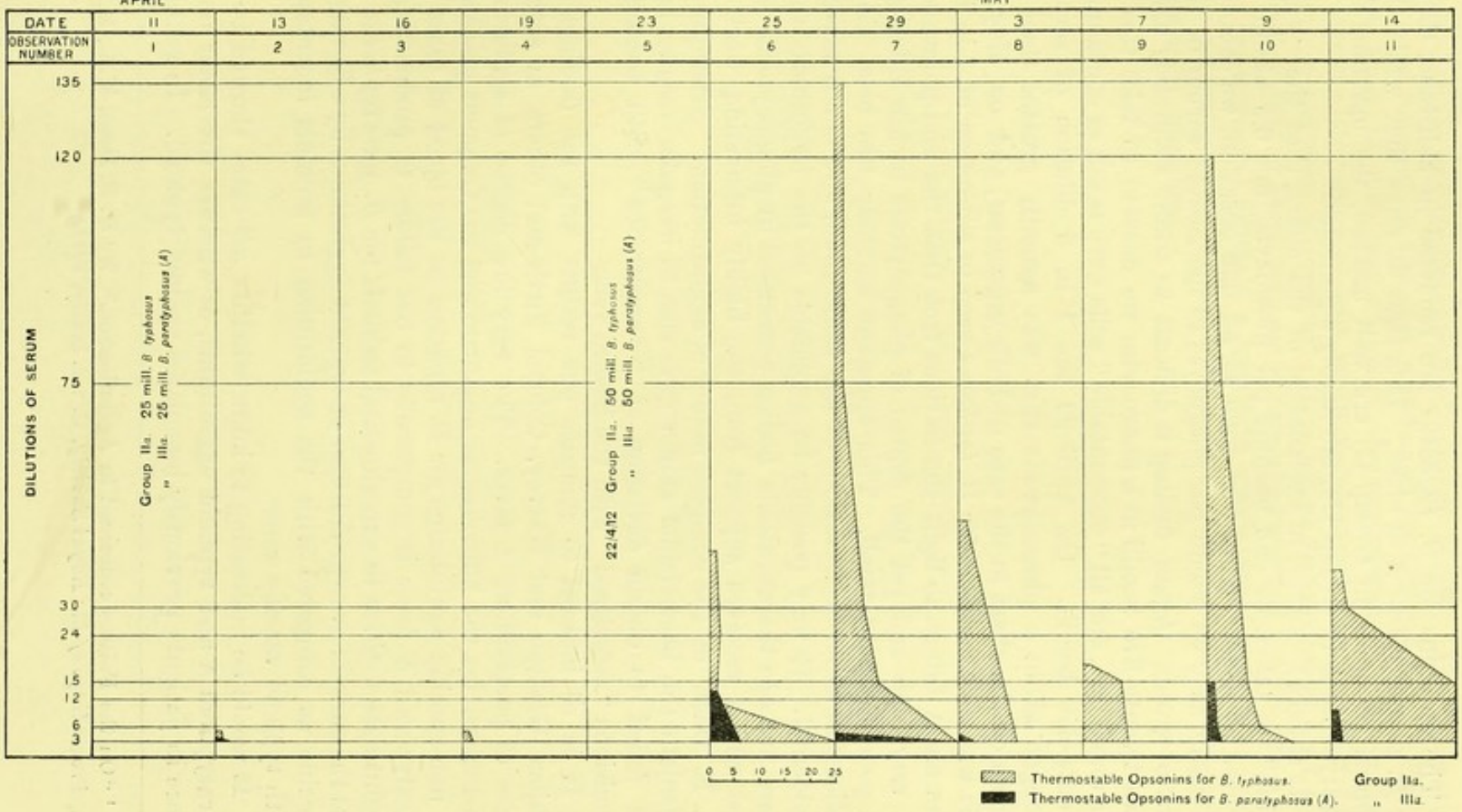


The agglutinin production is shown in Chart IV, and again the agglutinins for *B. typhosus* are much more marked than for *B. paratyphosus* A. The thermo-stable opsonins, calculated by Klien's[1] method of dilution to an end-point, are shown graphically in Chart V, where the number of bacilli per phagocyte is shown for each dilution by the horizontal measurement of the shaded area at each level, one degree of the scale corresponding to one bacillus per phagocyte.

[1] "The Opsonins in Typhoid Immunity." By H. Klien, M.D. *Johns Hopkins Hospital Bulletin*, June-July, 1907.

CHART V.

OPSONIC INDICES (Klien's Method of Dilution)



Each degree on the horizontal scale indicates one bacillus per phagocyte. The degree of phagocytosis in each dilution is shown by the horizontal measurement at each level.

The chart shows how much greater the phagocytosis becomes after immunization with *B. typhosus* than after immunization with *B. paratyphosus (A)*

The opsonins for *B. typhosus* are rendered in half-tone, those for *B. paratyphosus* A in black. It is seen at once that while the typhoid opsonins in Group II. are well marked, the opsonins for paratyphoid A in Group III. are hardly significant.

It should be added, in explanation of the chart, that the "end-point" is taken as one bacillus per phagocyte, not 0·5 bacilli as advised by Klein. This was done as, on one occasion, we counted as many as 0·98 bacilli per phagocyte in the heated serum of the control group. Again, finding it difficult to count accurately more than twenty-five bacilli in a phagocyte, we decided to take this as our maximum, and all "uncountable" cells were taken as containing twenty-five bacilli. On April 29 the 1 in 3 dilution of serum brought about a phagocytosis that was actually greater than is shown on the chart in the case of both organisms, and on May 14 this was also the case for *B. typhosus* even in a dilution of 1 in 15. The chart brings to light the curious fact that the end-point may be very high and yet the degree of phagocytosis in low dilutions be comparatively small. This apparent anomaly has been since noticed in other observations of the same kind, and is not merely accidental. It may possibly be explicable on the hypothesis that there are *two* thermo-stable bodies concerned in phagocytosis—an idea which receives support from the highly interesting work of H. R. Dean[1] on the complex nature of agglutination. Attempts to calculate the bactericidal action gave rise to irregular results, and we failed to obtain deviation of "complement" with bacterial emulsions "sensitized" with the heated sera.

It is of interest to compare our results with the findings of Majors Grattan and Harvey, Colonel Firth and others in actual cases of paratyphoid A fever. The very low degree of agglutinin production for the homologous organism and the frequent presence of non-specific agglutinins for *B. typhosus* in the blood of cases of paratyphoid A fever is comparable to our failure to produce high agglutination-*titres* in experimental animals for *B. paratyphosus* A, and the curious over-production of typhoid agglutinins by a bi-valent vaccine as compared with the agglutinins in animals inoculated with typhoid vaccine alone.

It would be interesting to know whether *all* cases showed both paratyphoid A and typhoid agglutinins, or whether the latter only arose in patients previously inoculated against typhoid. In rabbits

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[1] "On the Factors concerned in Agglutination." By H. R. Dean, M.A., M.B. &c., *Proceedings of the Royal Society*, B. vol. lxxxiv, 1911.]

immunized with paratyphoid A vaccine only we were unable to demonstrate any agglutination of typhoid bacilli. Another matter of great interest is the fact, mentioned by Colonel Firth, that no less than 14 per cent of cases of paratyphoid A fever become "carriers" for more or less protracted periods, as opposed to 2 per cent in the case of typhoid. It is tempting to connect this with the low *titre* of bacteriotropic substances recorded both in cases and in experimental animals, a condition which would appear to favour the permanence of bacterial foci in the tissues after the septicæmia has been overcome.

PREVENTION OF MALARIA AT HYDERABAD, SIND.[1]

By Major H. HERRICK.

Royal Army Medical Corps.

The following account of antimalarial measures undertaken during the year 1911, at Hyderabad, Sind, may be of interest to the casual reader of the Journal, and probably will be of special interest to officers who have been stationed there in former years.

The Cantonment of Hyderabad, Sind, is situated on a plateau about 30 ft. above the level of the surrounding country and[2] 134 ft. above sea level, in lat. 25° N., and long. 63° E. and about two miles from the Indus.

The plateau is composed chiefly of gravel and sand, with patches of clay; the surrounding country is mostly alluvial, flat, and cultivated where the soil is favourable. The low-lying country was flooded in former years by the annual rise of the Indus, which filled the irrigation canals and caused them to overflow their banks. This is most important from the point of view of antimalarial measures.

Climate.—There is a cold and a hot season. The former, lasting from November to February, has an average shade temperature ranging from 75° to 45°. The hot season, from March to October, is very trying; the temperature during the hottest months ranges from 110° F. to 115° F. and occasionally reaches a maximum of 120° F. in the shade.

The prevailing winds are from the south or south-west in the wet season, and from the north or north-east in the cold season.

Rainfall.—The annual rainfall is very slight, usually only a few inches.

City.—The native city and sudder bazaar are to the north and north-east of the cantonments, and about half a mile distant.

Water Supply.—The drinking water is taken from the Indus at Gidu Bandar, which is on the left bank of the river opposite Kotri. The supply comes to Hyderabad in a masonry aqueduct and is supplied to the cantonments from water-towers.

The water for irrigation purposes is supplied from canals which take off from the Fuleli Canal, the Fuleli taking off from the Indus

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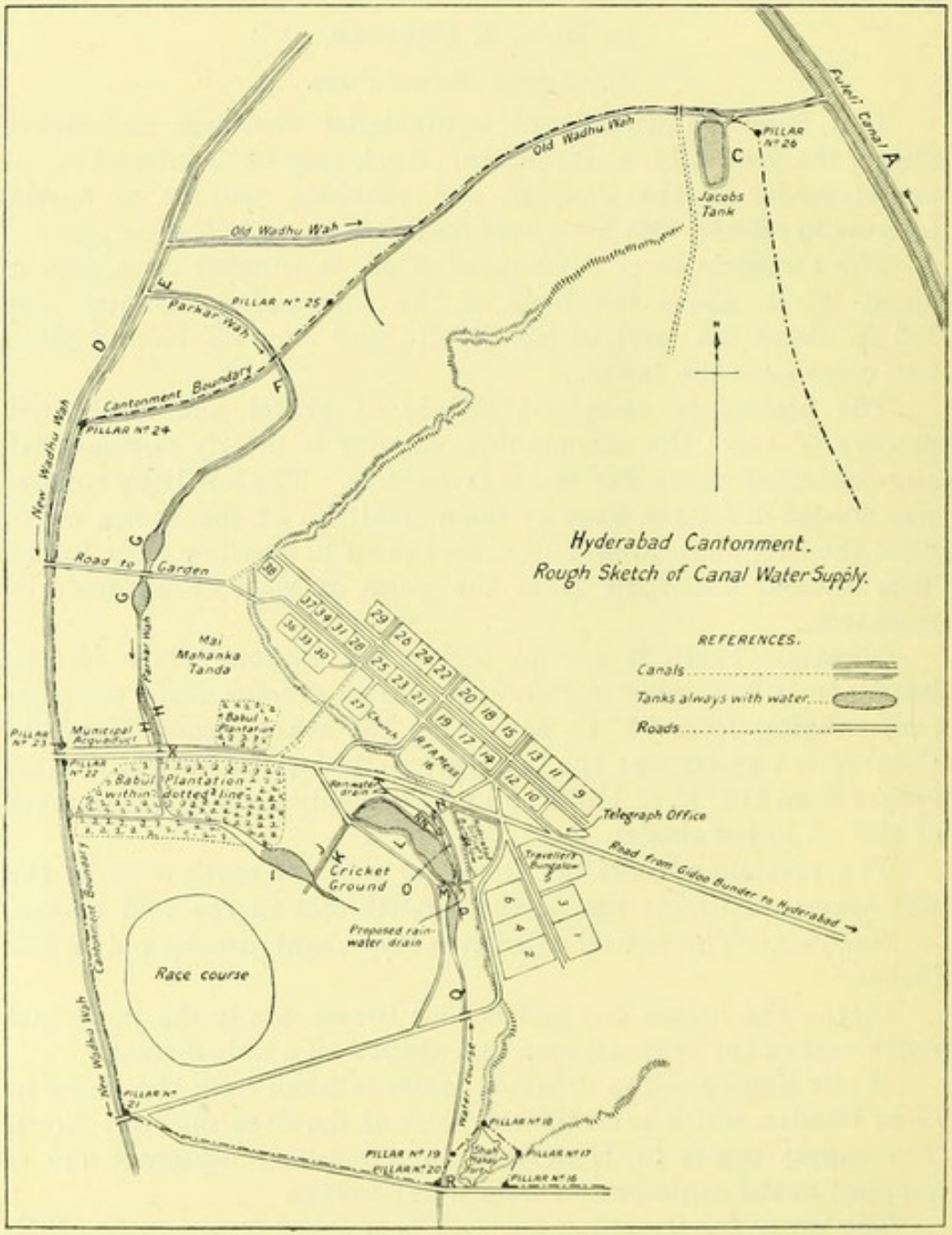
[1] Received for publication April 17, 1912.

[2] Authority, Intelligence Branch of the Division of the Chief of the Staff.

a few miles above Kotri. The Fuleli Canal is marked "A" on the map.



The New Wadhu Wah is the main canal at the west of cantonments; the old Wadhu Wah takes off from it, as shown on the map, and irrigates all the north of cantonments.

The Parkar Wah Canal takes off from the Wadhu Wah at the

sluice marked "E" on the map, and is the main irrigation canal to the west of cantonments. This canal, when allowed to overflow, filled up tanks marked "G" and "I" on the map.

Another canal, which is outside cantonment limits, took off from the New Wadhu Wah at boundary pillar No. 21 and flowed parallel to the road to boundary pillar No. 20, where there was a sluice marked "R" on the map, which was supposed to regulate the flow of water into the watercourse leading to tanks "P" and "L."

The ground to west of the above mentioned watercourse was irrigated by lifting the water by means of Persian wheels. Tanks "G" and "I" could also communicate with tanks "L" and "P."

The annual rise of the Indus is due to the melting of the snow at its source, and at the sources of its tributaries, and by the end of June, or beginning of July, the Indus, at Kotri, is in full flood, and the canals are rapidly filling, and by August in former years the west and south parts of the cantonment were one vast jheel, which, when partially dried, made an excellent breeding-place for mosquitoes. This was the state of affairs when I arrived at Hyderabad in September, 1909, for temporary duty, and also in November, 1910, when I was permanently transferred to the station. All the tanks were full, and most of the ground under water, and everywhere mosquitoes were breeding.

Nothing much could be done in 1910 as the canals had overflowed; so I had to turn my attention to what could be done next year to get rid of the water already in the tanks, to fill up these tanks, and to prevent more water getting in.

When I first suggested cutting off the water through the Parkar Wah Canal there was an outcry that the cantonment would lose revenue from stoppage of cultivation, and from the diminished sale of lac from the babul trees in the plantation to the west of the cantonment.

The first difficulty was got over by repairing sluice "E" and making the cantonment superintendent responsible for the supply to the cultivators, so that only a sufficient amount of water should be let in, and no more. By these means water was never left standing more than twenty-four hours in the irrigation channels.

As to the babul trees—a forest officer was consulted and gave as his opinion that once every two years was sufficient to water the plantations. So both these obstacles were surmounted.

The Parkar Wah was bunded at X, this stopped the water getting into tank "I." A bund was also put at the upper end of

tank "L" where it adjoins the road. The next step was to get rid of the water from tanks "L" and "P," and try to prevent them filling again. These tanks were the main breeding-grounds for anopheles mosquitoes, and were in close proximity to a highly infected native population.

The soldiers from barracks used to spend a great portion of their evenings fishing in these tanks, and I am certain that many men became infected while so occupied.

FIG. 1.—Tank "P." after completion of work. The water went beyond the trees. All the light portions of picture were formerly under water.

The Hyderabad Gymkhana Club and the Royal Field Artillery officers' mess were always full of mosquitoes in the evening, and bungalow No. 6 on the map went by the name of "Fever Hall." Bungalow No. 5, "the dak bungalow," was full of anopheles mosquitoes, and my wife and I got infected there shortly after our arrival at the station.

I was sitting one evening by the bank of tank "L" telling my troubles and propositions to a civil engineer, when the thought struck us, why not grade the banks of these tanks so that the flow instead of being from the south, as shown by arrow on the map,

should be from the north-west? Acting on this idea I ordered the sluice at "R" to be closed and kept closed, so that no water could get in, and then the filling of tank "P" was commenced, using jail labour.

We had many checks. First by being flooded by water from the municipal aqueduct, and again by the sluice at "R" being opened, by whom I could never find out. The sluice "R" was

FIG. 2.—Tank "L.," looking south, before grading and draining.

again closed, and a bund put there as well to absolutely put a stop to any more "regrettable incidents." The grading and filling of tank "P" was now continued, and in about twenty-seven days it was reduced to a narrow channel about 6 ft. wide with a fall to the south. I regret I did not take a photograph of this tank before commencing work, but give one of the tank after the filling was completed. In this photograph the channel is well shown.

Tank "L" was next attacked. In addition to jail labour we

were permitted by Lieutenant-General H. C. Slater, C.B., commanding 4th Quetta Division (who always gave us valuable assistance in all antimalarial and other sanitary measures), to employ the men of the detachment 1st York and Lancaster Regiment and of the 10th Jats, the European troops working two days a week in the mornings, and the Indian troops working four days a week in the evenings. They all worked with a will, so much so that, after eighty-one days' labour, tank "L," the Beyla tank of Hyderabad, was dry, and one of the show spots for a general

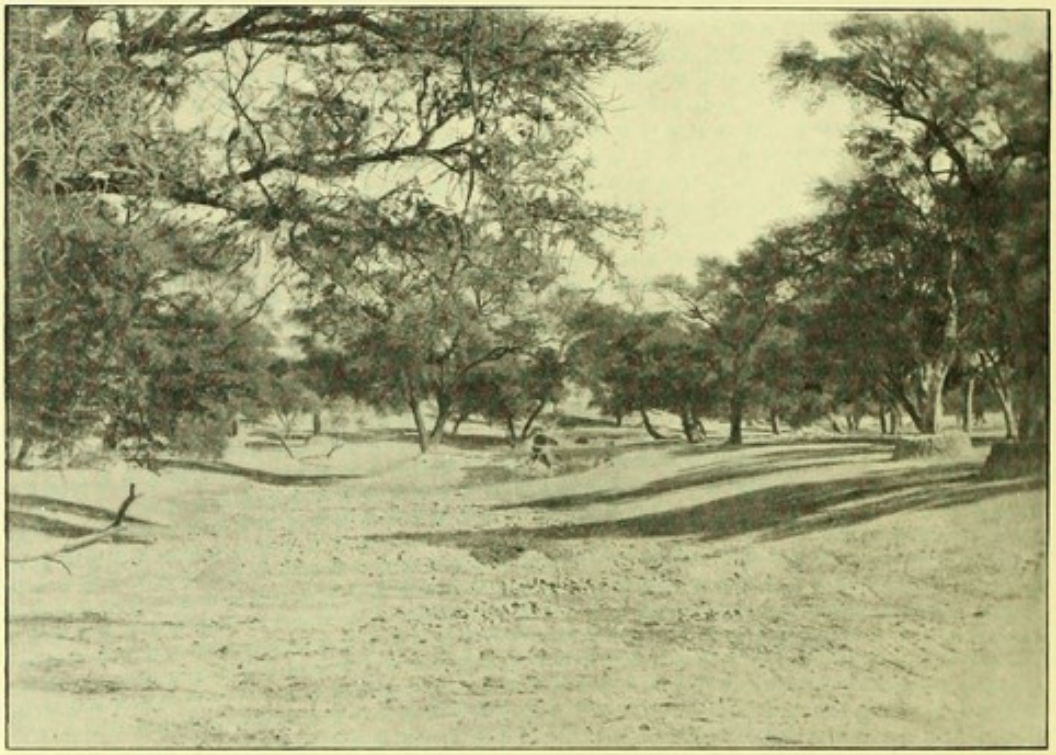


FIG. 3.—Tank "L.," after grading and filling, looking south.

inspection was gone for ever. I give photographs showing this tank both before and after filling. The first shows the tank used as a swimming bath, and the second shows the tank dry. As matters now stand all the tanks that are shown on the map as "holding water always," and consequently breeding mosquitoes, are dry. If water gets into tank "L" from an exceptionally high Indus, all that will have to be done will be to open the sluice at "M" and the water will flow south, and can be lifted by Persian wheels, which were previously used for irrigation, and the place will be left dry in less than a week. Various estimates were put in by experts for the

work of filling the tank or for getting rid of the water otherwise. I heard sums from 1½ to 2½ lakhs of rupees mentioned. That the water should be drained back to the Indus, by means of locks, was another wild suggestion.

The Hyderabad Cantonment Committee were able to do the work for 1,500 rupees.

Hyderabad, Sind, had never been considered a health resort, but its evil reputation was almost entirely due to the incidence of malaria. The following table will give an estimate of the sickness generally.

	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911
Average annual strength	390	458	483	497	494	411	492	488	443	523	556
Admissions—all causes	479	610	526	393	474	550	516	586	411	418	282
Ratio per 1,000, average strength, all causes	1,228	1,311	1,089	790	959	1,338	1,049	1,201	928	799	507
Average constantly sick—all causes	26·34	27·5	29·92	27·34	28·24	20·79	19·16	19·94	16·48	18·75	14·11
Admissions for malaria	183	164	214	114	69	274	300	299	177	165	84
Malarial ratio per 1,000 average annual strength	469·2	358·1	443·1	229·3	139·6	666·6	609·7	612·7	399·5	315·4	151
Average constantly sick—malaria	6·28	3·71	6·13	4·73	2·38	5·21	8·04	7·60	5·11	7·03	2·74

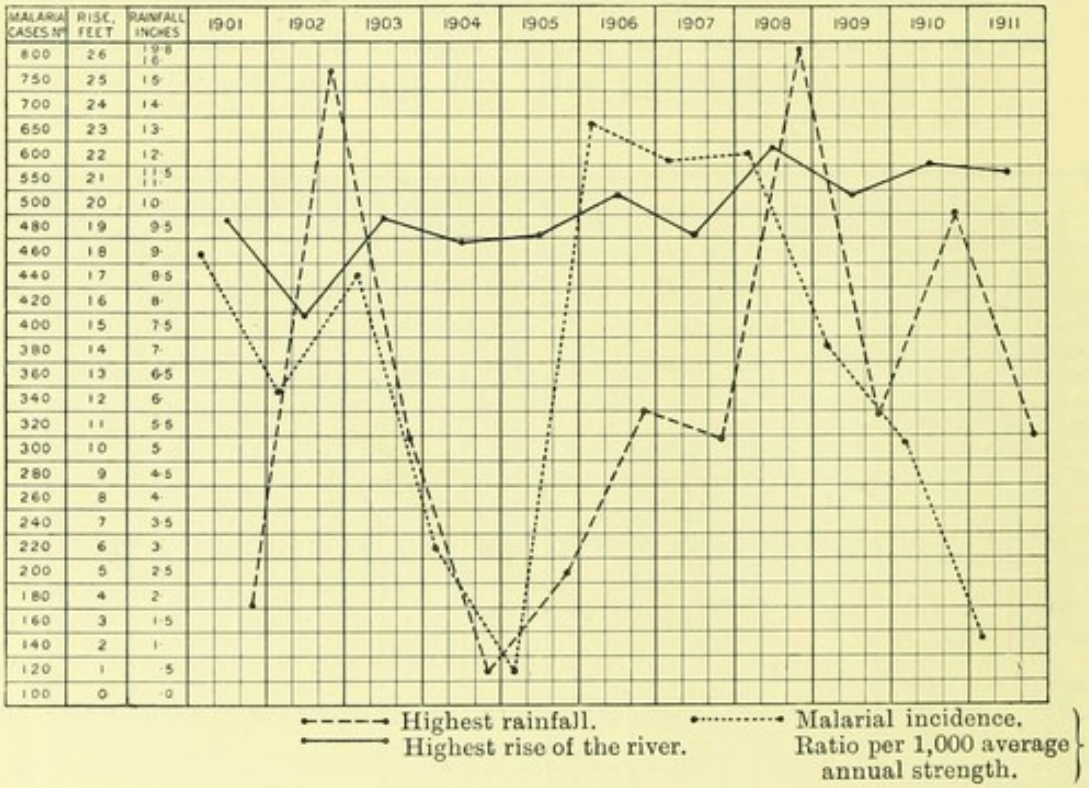
Remarks.—1905 was the first year in which men were treated in barracks.

In 1910 there was an apparent increase in the average constantly sick from malaria; this was due to keeping men suffering from benign tertian in hospital for ten days after the temperature became normal, and malignant tertian cases till all parasites had disappeared from the peripheral blood.

The rainfall at Hyderabad does not appear to have any influence on the incidence of malaria, but the rise of the River Indus distinctly *has*, especially in some years, as can be seen from the attached chart. A high river means bursting of bunds and much flooding. This was most marked in the years 1901, 1903, 1906, 1907, and 1908.

Probably the large number of admissions for malaria in 1906

was due to two units arriving from very malarious stations, the 32nd Battery R.F.A. from Deesa, and a detachment 1st South Wales Borderers from Mian Meer, and also to an exceptionally high flood in the Indus, which inundated the surrounding country and left many breeding-places for mosquitoes.



When the fundamental work of getting rid of mosquito breeding-places in cantonments was completed, we turned our attention to getting rid of adult mosquitoes in the houses, and began on the old huts in the Indian infantry lines, which were about to be demolished prior to the erection of new barracks. These huts were of mud, with *kutcha* roofs, smoke blackened inside—an ideal hiding-place for mosquitoes. Sulphur was used to fumigate them, 2 lb. being burnt for each 1,000 cubic feet of space. This must have killed all the mosquitoes, as, previous to fumigation, larvæ were constantly found in puddles left by workmen in the lines, but after fumigation larvæ were never found. The *débris* from the old huts was used to fill in hollows and level the ground in the vicinity of barracks. Levels were taken, and the work done in a scientific manner. The new barracks, Indian infantry lines, were then fumigated, and lastly the British troops barracks.

These British barracks, seven (7) in number, are very large rooms, each having a capacity of 100,000 cubic feet, and three large ventilators in the roof, besides many doors and clerestory windows. The ventilators in the roof were covered with tents, the doors and the clerestory windows closed and made as air-tight as possible by pasting brown paper over the chinks. The same amount of sulphur was used—i.e., 2 lb. per 1,000 cubic feet, and three hours allowed for fumigation. Everything living in the rooms was killed, including insects, birds and reptiles.

Prophylactic Issue of Quinine.—As many adverse opinions have been expressed as to the value of quinine as a prophylactic, I determined to give it a fair trial, but, owing to the stringent orders in the 4th Division about the issue of quinine to all men, I was unable to leave any men as a "control," hence the value of the trial is largely negatived.

Fifteen grains of quinine were given on two consecutive days to all European troops, commencing early in July. The quinine was given in solution with dilute nitro-hydrochloric acid, which is a valuable liver stimulant. After about a month I stopped the nitro-hydrochloric acid and gave dilute hydrochloric acid, which does not upset the digestion (as the other dilute mineral acids do), and hastens absorption.

The quinine was given between breakfast and dinner, usually about 11 a.m., and always under the close supervision of an officer, Royal Army Medical Corps. I mentioned my method of giving the prophylactic quinine to the P.M.O. 4th Quetta Division, Colonel W. G. Macpherson, when he made his inspection at Hyderabad on August 10, 1911, and he evidently found this method of administration with hydrochloric acid useful as he issued a circular letter on the subject, dated Quetta, September 22, 1911, saying "that when salts of quinine are given prophylactically. . . . they must be dissolved by hydrochloric acid in very dilute solutions."

To be of any use, quinine (given as a prophylactic) must be commenced some months before an epidemic is expected, given in sufficient doses, and under careful supervision, care being taken that no man escapes.

The followers, syces, &c., were also given quinine as a prophylactic by a sub-assistant surgeon attached to one of the Royal Field Artillery units.

Quinine was also given to all officers' servants and their families in cantonments; the drug was taken round and administered by men of the cantonment mosquito brigade, under an Indian N.C.O.

To malarial patients in hospital quinine was given in 10-gr. doses, if possible two hours before an anticipated attack in benign tertian cases, and then after the temperature became normal in 5-gr. doses, three times a day, for ten days.

In malignant tertian cases 10-gr. doses were given two or three times a day according to the severity of the attack. The after-treatment of malarial cases was carried on for four months. Each man on admission with malaria was given a malaria case-sheet, a copy of which is attached ; and on this sheet was entered the total amount of quinine given. When a man was transferred the malaria case-sheet went with him to ensure continued treatment. Ten grains of quinine daily was the dose given in the after-treatment of an attack, and, if a relapse occurred, the fact was noted on the sheet in red ink, and the patient began anew his four months' curative course. A register by units was also kept in hospital, in which was noted the daily attendance of the men undergoing continued treatment.

Acid hydrochloride of quinine was the salt always given in hospital and quinine sulphate to those attending hospital.

A good deal of the above plan of treatment may be well known to officers who have served in the 4th Quetta Division, but I give it for the benefit of those who have not been so fortunately situated. All cases undergoing treatment in barracks were segregated as far as possible, but as the Government has not yet seen fit to provide men with mosquito nets, the isolation of men, except in those units which provided nets out of regimental funds, was merely nominal.

A step forward has at last been made, as, by an Indian Army Order just published, I see that units can purchase nets for Rs. 3·2 from the Army Clothing Department. The size of the net is 6 ft. 6 in. by 3 ft. 6 in. by 4 ft. I only hope that the net will be made without an opening in the side.

One corps only at Hyderabad, namely, the detachment 1st York and Lancaster Regiment, provided the men with nets, but as they had not got these nets in September and October, 1910, they were heavily infected. The Artillery Brigade arrived from South Africa after the season of greatest prevalence of mosquitoes, and was not so severely infected.

Nets are provided in hospital for all men suffering from malaria, and the malaria ward is also screened by gauze doors, and all windows are covered with wire gauze ; in the hot season when punkahs are in use the nets can be removed. I have recommended that if the malaria at Hyderabad continues, the barrack rooms

be screened, but I think the cost will be prohibitive. Failing this I recommended that a portion of the verandah of each barrack room should be made mosquito proof with wire gauze, to segregate all men undergoing their four months' treatment.

The average monthly incidence of malaria for the last nine years, as well as for 1911, is shown in the following table :—

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Average last 9 years	17·1	4·0	8·11	11·44	12·0	10·44	9·77	9·88	11·66	18·88	43·0	37·77
1911 ..	12	8	6	11	17	11	2	2	4	5	3	3

This table shows that, in previous years, the last three months of the year accounted for the greater number of the annual admissions for malaria, that is just after the breeding season of the anophelines, with probable fatigue and cold as an exciting cause. An alternative theory to the above, which I give for what it is worth, is that when the wind changes to the north or north-east, which it does in November or December, the infected mosquitoes get blown into the lines from the city and sudder bazaar. Again a slight rise in the number of malarial attacks is seen on the commencement of the hot weather, which may be due to the awakening of infected hibernating mosquitoes, or the onset of the warm weather may be the encouraging factor.

Relapses.—Relapses, I take it, are due to a decrease in the germicidal power of the blood, or to a diminution in antitoxic power to a toxin which is produced by the malarial parasite. Relapses always occurred in the weakest men who never played games, and did little else than loaf around barracks.

I examined 108 malaria case-sheets, and found a record of relapses in 33, or 30·55 per cent.; every slight rise of temperature was taken as a relapse, even when a man was only detained for an afternoon, and a blood examination was always made. In only nine cases out of 108, or 8·33 per cent., was the blood of a patient with a relapse found to contain parasites—out of those nine cases in which parasites were found, eight were malignant tertian, one was benign tertian.

The average time of apyrexia in malignant tertian cases which relapsed under treatment was twenty-eight days, though in one case the apyrexia was as short as fourteen days, and in another as long as three and a-half months. In the benign tertian case which relapsed, and in which parasites were found, twelve days was the time of apyrexia.

Of the 33 relapses, 14 were malignant tertian; 19 were benign tertian.

The average time under treatment before relapse was: malignant tertian cases, 45·85 days; benign tertian cases, 36·42 days.

Cases with more than one relapse: malignant tertian, 3; benign tertian, 4.

Number of smears examined for parasites from January 1, 1911, to December 31, 1911, was: malignant tertian, 27; benign tertian, 35; negative, 22; not differentiated, nil.

Recognizing the importance of ascertaining the degree of infection of the European troops, it was decided to examine the blood of all men in the station. Five minutes was allowed for each slide, and opposite each man's name in the list was put the result, thus: five minutes ± (James). Twenty slides were examined in a day—more could not be undertaken, as routine work could not be neglected, and the climate was not conducive to prolonged microscopical investigation.

Months of Greatest Incidence of Mosquitoes.—The months of greatest incidence of mosquitoes were, in my experience, from September to November, though other officers have noted March and April.

The anopheles mosquitoes found were: *Myzomyia culicifacies, M. Rossi, Ch. pulcherrima, Pyrethophorus jeyporiensis.*

Captain J. Anderson, I.M.S., found *Ch. pulcherrima* breeding in the Beyla tank—tank "L" on the map.

Captain F. C. Fraser, I.M.S., wrote to me as follows: "The Beyla tank 'L' and a tank the other side of the city always harboured larvæ of *Ch. pulcherrima*. Jacob's tank to the north of cantonments was sterile (chiefly because it grew no weed like the two former tanks). I always noticed that when the water rose in the Beyla and submerged the weed, the larvæ grew scanty, or disappeared; as soon as the water sank to the level of the weed, the larvæ began to teem. In several bungalows in Cantonments, and once in the Jail gardens, in small pools due to pipe-water waste, or watering, larvæ of *P. jeyporiensis* were found. These were the only two kinds I ever detected, and they were never found together; one kept to the tanks, and the other to the pools, and both are mosquitoes with an evil reputation for carrying malaria, especially *Ch. pulcherrima*. Colonel Adams, R.A.M.C., sent me a mosquito caught in his house which proved to be *P. jeyporiensis*, and I have taken both kinds in the club wash-house."

I lived in Colonel Adams' bungalow, mentioned above, while at Hyderabad, and I hardly ever saw a mosquito in it, though it is

only 200 yards from a small native village, which must swarm with mosquitoes. I know the children living in this village have a high spleen index, though I regret I was never able to make a reliable census. Of all the European troops I examined, I only found enlarged spleens in 5·12 per cent.

The Club is within 100 yards of the Beyla tank "L," which was the chief breeding-place in cantonments, as mentioned before. A small tank marked "NN" on the map gave no trouble, as it was not deep, being merely a depression where rain water, and water from the swimming bath, used to collect. It was graded and filled in the same manner as tank "L." Many complaints were made to me that I was spoiling the only green and picturesque spot in Hyderabad, and also killing all the grass on the cricket ground; all this may be true, but I, for one, prefer an arid waste to malaria. As far as watering the cricket ground goes, water can be found about 20 ft. below the surface, and it should be quite easy to sink a shallow well and pump up sufficient water for irrigation by means of a windmill.

The breeding-places of mosquitoes in officers' compounds gave a certain amount of trouble until I abolished all catch-pits and had the bath waste run into gardens with quick-growing plants, such as canna and guinea grass, in them. Funds did not admit of a mosquito brigade being kept up for the officers' lines as well as for the Sudder Bazaar, so I arranged that the sanitary orderly of each unit shouid visit the officers' bungalows of his own corps, and report to me if any insanitary condition was found. I found this plan to work well.

And now to compare the admissions for malaria for 1911 with that of the previous year :—

Malaria Cases by Months.

1910.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	20	12	2	10	11	3	6	6	3	14	38	40

Ratio per 1,000 *Average Monthly Strength.*

Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
32·62	20·16	3·35	17·33	18·90	5·10	10·60	10·86	10·38	60·60	73·78	69·56

Malaria Cases by Months.

1911.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	12	8	6	11	17	11	2	2	4	5	3	3

Ratio per 1,000 *Average Monthly Strength.*

Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
20·54	13·55	10·61	20·67	30·96	19·89	3·77	3·81	7·39	8·71	5·35	5·23

Malaria Cases by Months.

1912.	Jan.	Feb.
	3	4

Ratio per 1,000 *Average Monthly Strength.*

Jan.	Feb.
4·35	7·11

Officers. 1910		*Officers.* 1911	
Strength	13	Strength	15
Malaria cases	6	Malaria cases	2*
Ratio per 1,000	461·53	Ratio per 1,000	133·33

* One officer admitted twice.

Women. 1910		*Women.* 1911	
Strength	33	Strength	39
Malaria cases	6	Malaria	*Nil.*
Ratio per 1,000	181·86		

Children. 1910		*Children.* 1911	
Strength	47	Strength	62
Malaria cases	6	Malaria	*Nil.*
Ratio per 1,000	127·65		

Pyrexia of Uncertain Origin.

1910	1911
2	2

Sand-fly Fever.

1910	1911
20	*Nil*

One officer remarked to me that my figures were valueless because of their paucity. My reply to all who are of a like mind is, that I do not aspire to be a compiler of statistics, I merely state facts and figures as they occurred at Hyderabad. I regret that opportunity did not offer of ascertaining to what extent the mosquito population was infected.

Much still remains to be done, namely, to find out the percentage of mosquitoes with zygotes in their stomachs and sporozoites in their salivary glands, also the seasonal variation in infectivity.

One of the Royal Army Medical Corps officers, now stationed at Hyderabad, is being trained at Amritsar in special malaria work, and he will be able to afford valuable assistance in further antimalaria work at this station.

I cannot conclude without thanking the following officers and warrant officers for valuable assistance, without which I would have been unable to carry out the work that was done in Hyderabad in 1911: Captain G. W. Mortimer, 10th Jats, the Cantonment Magistrate; Captain J. A. S. Phillips, I.M.S., the Cantonment Medical Officer; Lieutenant W. B. Rennie, R.A.M.C., in charge

of malaria wards; Lieutenant K. G. S. MacQueen, and Assistant Surgeon J. S. Menezes, I.S.M.D., who compiled the greater part of the statistics for me, and kept the various records.

MALARIA CASE-SHEET.

Instructions.

A malaria case-sheet will be kept for each man who has an attack of malaria during the year.

A separate roll of these men will be kept for each regiment and battery.

All cases should be admitted to hospital.

After discharge from hospital each case should be treated with quinine for at least four months.

Especial care should be taken in the case of men suffering from malignant tertian infection, to ensure that they do not return to barracks while crescents are to be found in the finger blood.

No man should be struck off the roll until four months have elapsed since the last manifestation of the disease, during which time he has been under continuous quinine treatment.

NAME NO. AGE SERVICE IN INDIA

REGIMENT OR BATTERY COMPANY OR SQUADRON.

(1) Particulars of first attack in 191–, *e.g.*, clinical and microscopical features, treatment, &c.

ADMITTED DISCHARGED

(2) Whether fresh infection or recurrence of old infection?

(*a*) If fresh infection, give dates showing where probably acquired,

(*b*) If old infection, give particulars of previous attacks, *e.g.*, dates, variety of parasite found, &c.

Treatment.

(Any recurrences that may occur during this course of convalescent treatment will be entered as they occur, in red ink, giving full details.)

Date	Treatment and remarks

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Journal
of the
Royal Army Medical Corps.

Original Communications.

SOME OBSERVATIONS ON METABOLISM IN CONNECTION WITH AN EXPERIMENTAL MARCH.

By Colonel C. H. MELVILLE, Lieutenant-Colonel W. W. O. BEVERIDGE, D.S.O., and Captain N. DUNBAR WALKER.
Royal Army Medical Corps.

In August, 1910, on the second of the experimental marches described in this Journal (vol. xvii., 1911), an attempt was made to arrive at some idea of the nitrogen balance, and some other points, in those participating in the experiment. Naturally, it was only possible to do this in the case of a few individuals, and the three medical officers were selected for the purpose. These are referred to in the tables and plates as Nos. 1, 2 and 3.

The observations taken were as follows (see Tables I, II and III): weight, energy value of food (approximate), nitrogen in food (approximate), amount of fluid drunk, amount of urine passed, nitrogen in urine and nitrogen in fæces.

The energy value of the food, and the nitrogen in the food, were estimated as follows: The ration served to each officer was carefully weighed on issue, and as far as possible any article remaining unconsumed at the end of the twenty-four hours was also weighed. As far as concerns the biscuits, sugar, jam, tea, pepper, salt, and cheese, the figures may be taken as absolutely accurate. In the case of the meat, vegetables, and oatmeal absolute accuracy was impossible. The total amounts issued were accurately known, and

also the total amount uneaten (as a matter of fact an almost negligible quantity). These articles were, however, pooled for messing purposes and the actual proportion eaten by each officer

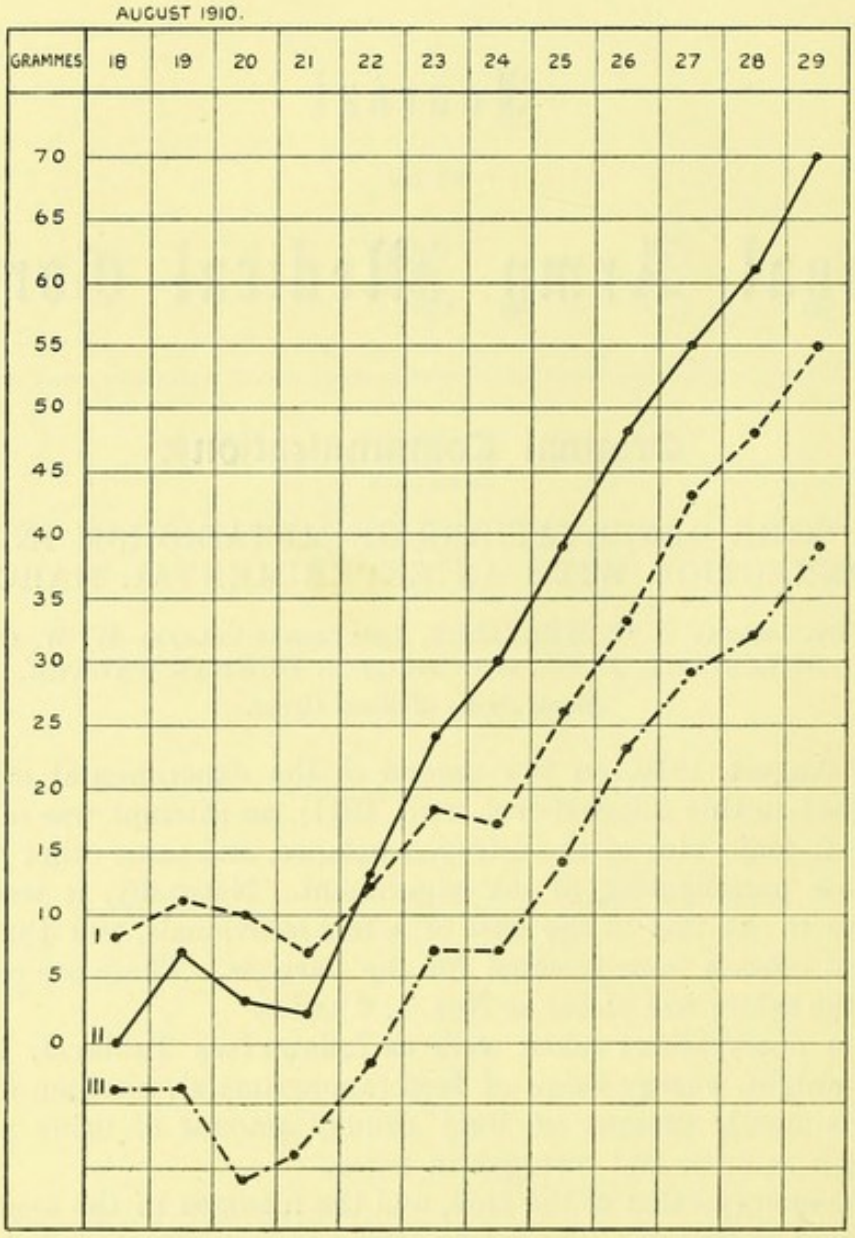

CHART 1.

was of course only approximately arrived at. The greatest care was taken to equalize portions and probably, on the whole period, the error in any one case was not very great. It must be admitted, however, that the figures for "nitrogen in food,"

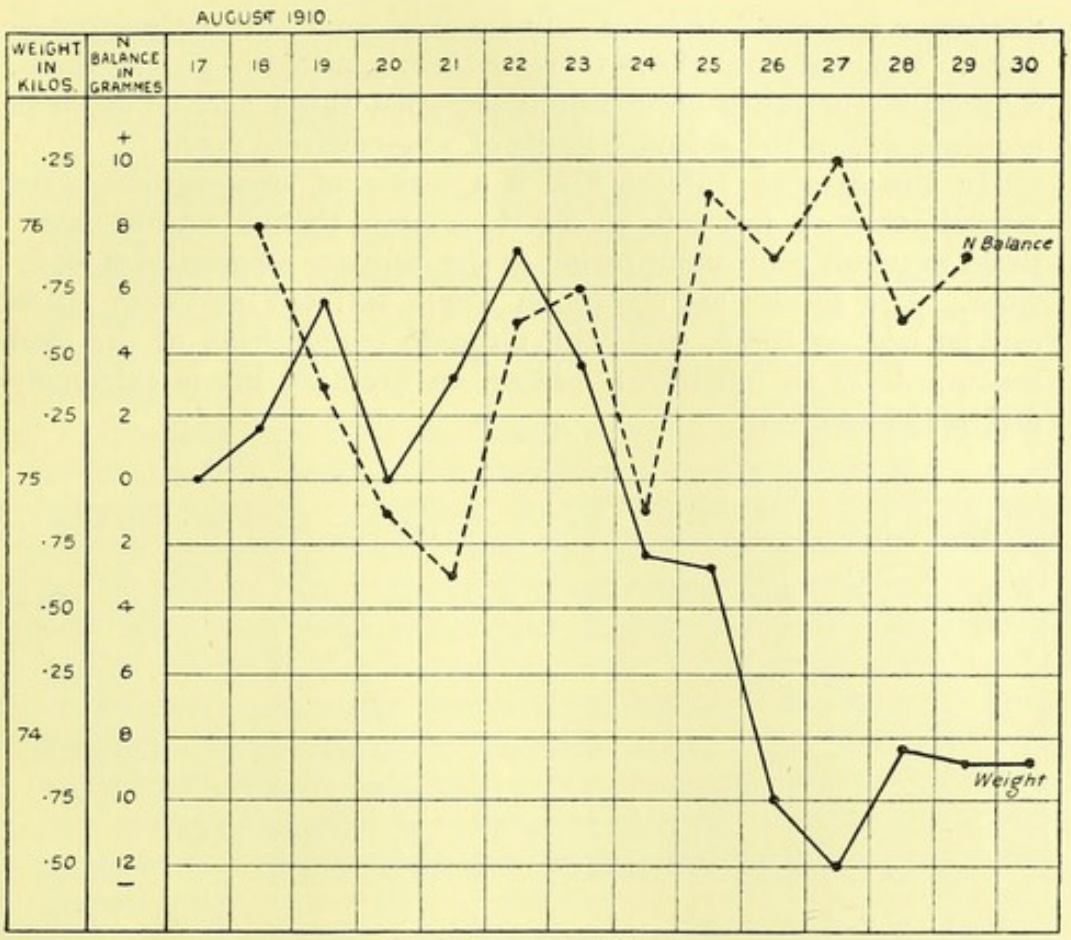

CHART 2. Subject No. 1.

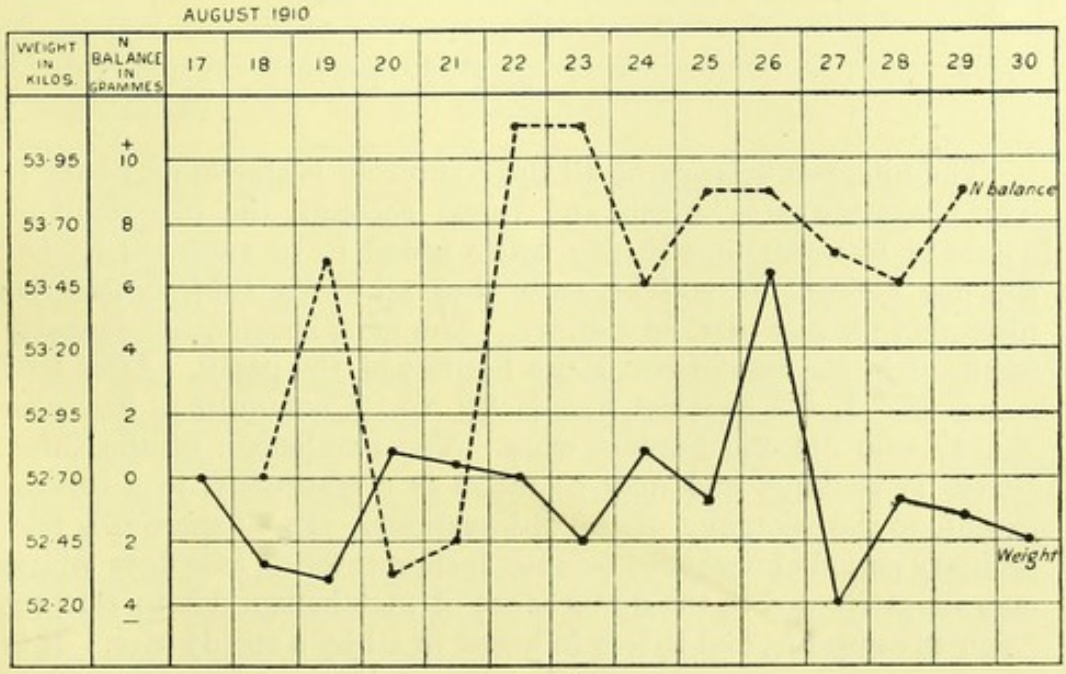

CHART 3. Subject No. 2.

"nitrogen balance," and "energy value of food" are only approximate. Decimal points have therefore been omitted in stating these. As regards the other observations recorded these may be taken as accurate within the ordinary limits of observational error.

In the case of subject No. 3 a series of observations were carried out for a few days before the march, though unfortunately these were not continuous owing to the ordinary exigencies of daily work. The results are shown on Table III, but not much stress can be laid on these, since the ultimate composition of the food consumed by an individual leading an ordinary life is extremely difficult to ascertain.

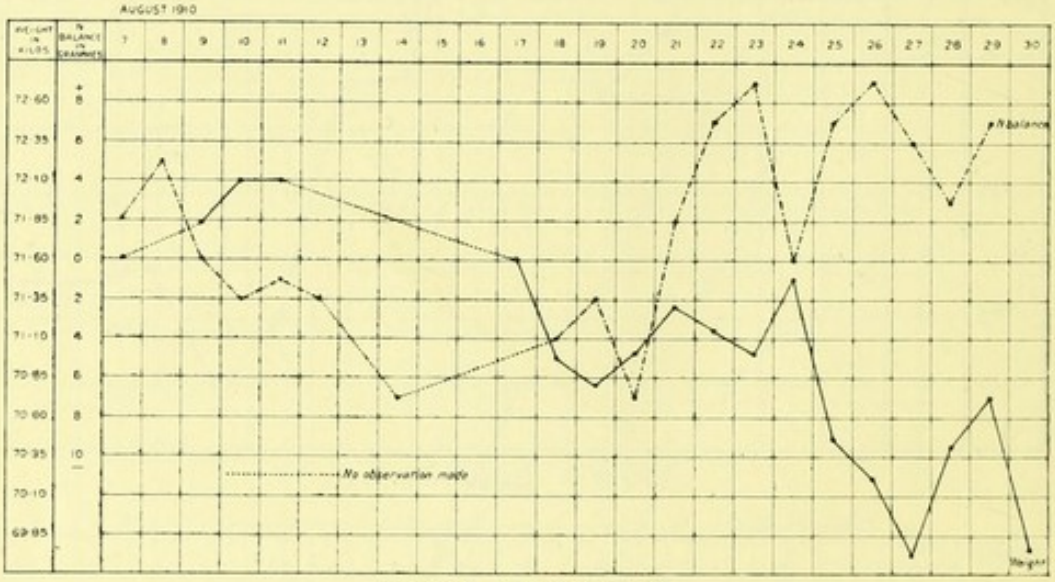

CHART 4. Subject No. 3.

The nitrogen balance of all three subjects is shown on Chart I. This chart shows a "Profit and Loss" account, the increment or defect of nitrogen in any day being added to or subtracted from the day before. It will be seen that all three subjects laid on nitrogen in a very marked manner. The most conspicuous increase occurred in the case of No. 2, the lightest of the party. After him comes No. 1, the heaviest, and lastly No. 3, not quite as heavy as No. 1, who did the hardest work. The parallelism of the three curves is striking, and may be taken as a testimony, to a certain extent, to the accuracy of the observations. In all there is a preliminary period of unsettled balance, lasting three or four days which may be attributed to the disturbance of metabolism due to settling down to camp life, and this is followed in all by a steady rise. It is

interesting to notice that this rise is checked in the case of Nos. 1 and 3 on the same day (24th), on which day also No. 2 shows the smallest increment occurring during this period. On this day occurred the change from tinned meat to fresh, a change which was accompanied by a sudden fall in the estimated nitrogen intake for the day. Since the calculations for intake and output were entirely independent of each other, this coincidence tends to confirm the accuracy of the former calculation. If the decreased intake had been due to under-estimation an increased rise would have been shown in the N. balance.

It will be seen from the Charts Nos. 2, 3, and 4, that there was no connection between the variations in weight and those in the nitrogen balance. Subject No. 1, the heaviest, lost 1·1 kilos; No. 3, somewhat lighter, who did much harder work, 1·9 kilos; whilst subject No. 2 practically maintained his weight, the total fall being only 0·25 kilo, occurring practically in the last four days.

If we assume that the nitrogen retained was utilized in the form of muscular tissue, we see that the true loss of weight in all cases was in reality somewhat greater than the above. Multiplying the nitrogen retained by 6·25 to bring it up to protein, and this again by 5 to represent muscle, we see that the total loss of tissue amounts to :—

In the case of No.	1	..	1,100	+	1,718 grm.	=	2,818 grm.
,, ,, ,,	2	..	250	+	2,187 ,,	=	2,437 ,,
,, ,, ,,	3	..	1,900	+	1,218 ,,	=	3,118 ,,

The work done was calculated in the case of No. 3 on the same basis as that used for calculating the work done by the men of the party (see this Journal, vol. xvii, 1911, p. 535), since this officer carried the same weight as they, and performed all the marches with them. Taking the entire difference between the work done and the energy value of the food consumed over the whole period, we find that there was in the case of this subject an estimated deficit of 9,634 calories. This might be taken as representing a loss of a little over 1,000 grm. of fat. The remaining loss (2,000 grm. roughly) was probably due to loss of water.

On Charts Nos. V, VI, and VII are shown: (1) The total amount of fluid consumed; (2) the same minus the urine passed; and (3) the variations in weight. The amount of fluid actually drunk was carefully measured at the time, and the urine was collected for twenty-four hours and measured. These amounts are therefore exact. The amount of fluid present in the food consumed

is approximate, but there is only room for a comparatively small error here as regards the relative amounts consumed on different days,

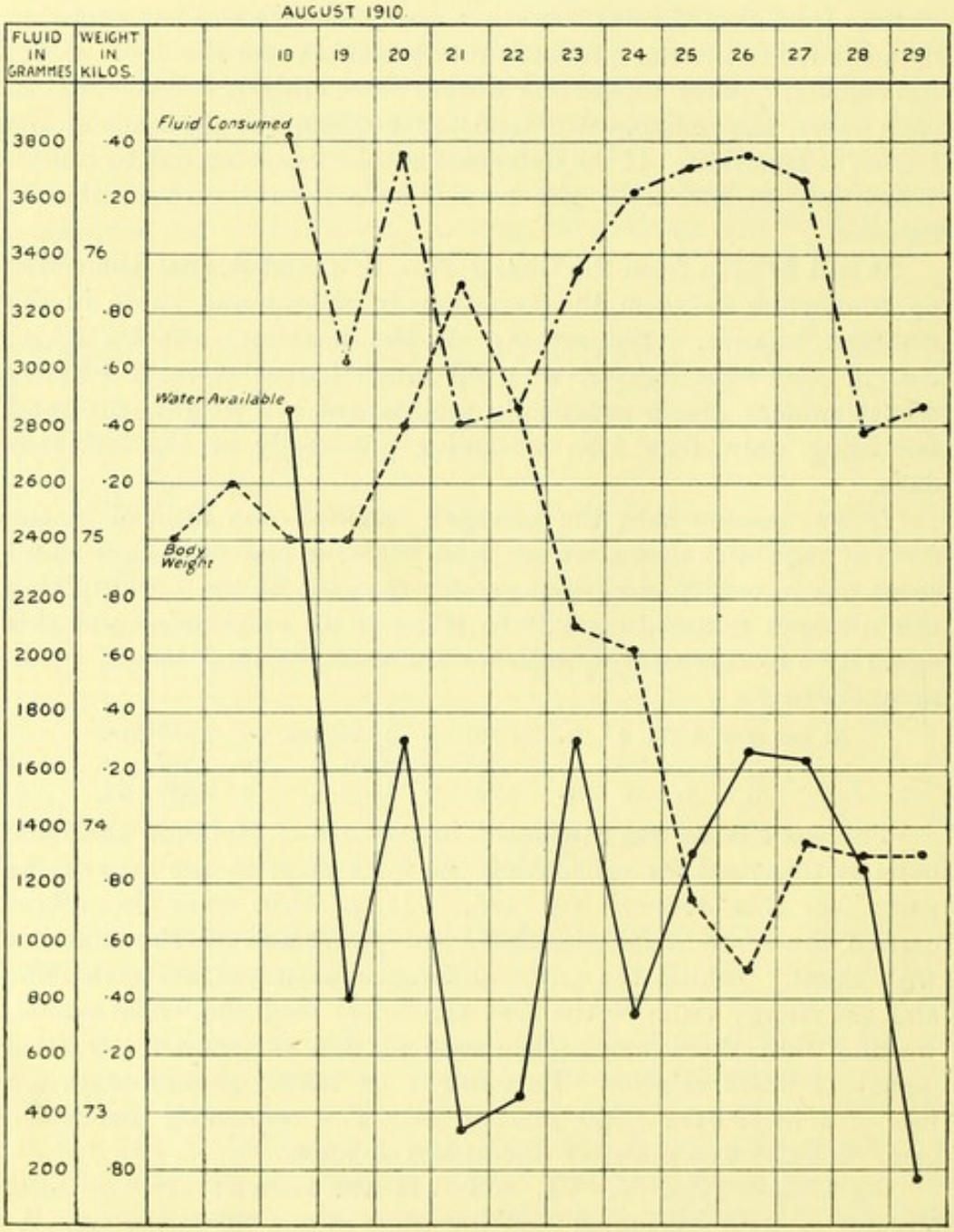

CHART 5. Subject No. 1.

in fact, the error is probably negligible. No soups, stews, or hashes were eaten, the food being cooked, except in the case of porridge,

without added water. In the case of the preserved meat this was minced, made into rissoles and fried with bacon fat. The fresh meat was always roasted. Accordingly, though the absolute amount of water ingested on any particular day can only be approximately stated, the relative amounts taken in in this manner on different days are fairly accurate. The amounts allowed are 850 c.c. per diem, on the 10th, 19th, 20th, 24th, 25th, 26th, and 27th, on the first three of which days fresh vegetables and on the

CHART 6. Subject No. 2.

remainder a full meat and fresh vegetable ration were given; 650 c.c. on other days.

In addition to the water actually consumed it is necessary to take into account that resulting from the combustion of the hydrogen of the food. According to van Noorden this may be taken as equivalent to 12 grm. per 100 calories furnished by the food metabolized. The actual energy expenditure is only known (approximately) in the case of subject No. 3. It varies from 3,000 as a minimum, to 4,631 as a maximum, with an average of 4,146. The amount of

water to be credited to this subject on this account would therefore range from 360 to 555 grm. with an average of about 500 grm.

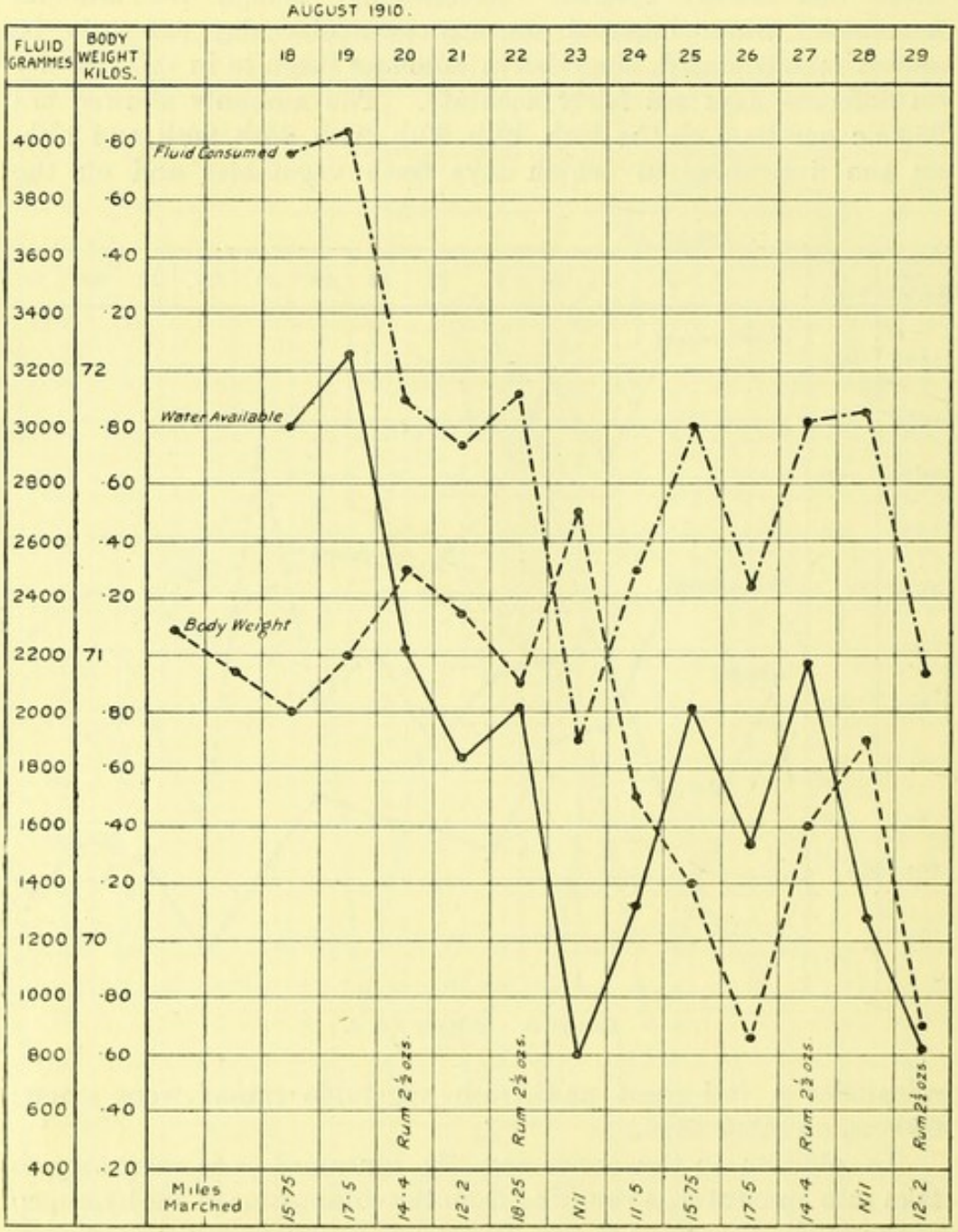

CHART 7. Subject No. 3.

The variations from day to day were comparatively slight, and not sufficient to affect seriously the general trend of the curve given in Chart VII. These figures have therefore been omitted.

The difference between the total fluid consumed and that passed as urine represents the amount available for respiration and perspiration, and should, it might be anticipated, vary directly with the weight. That is to say, that if a small amount of water were available on the 18th, the weight on the morning of the 19th would show a fall. In the charts the curve showing the weights is a day in advance of that showing the "water available," and it will be noticed that, though the curve for weight follows that for water available, there is a distinct lag in the correspondence. A fall or rise in "water available" is followed by a fall or rise in the weight, not on the next day, but on the day after that. The correspondence is closest in the case of subject No. 3, who did the most work. Thus the fall in "water available" on the 20th is followed by a fall in weight, shown on the morning of the 22nd. On this day the former curve shows a rise, followed by a similar change in the weight recorded on the 24th. There is a great deficit of "water available" on the 23rd, and this is followed by a marked fall in weight lasting till the morning of the 27th. The subsequent rally in weight on the 29th might be attributed to the considerable excess of "water available" on the previous three days. Here again the fall in the "water available" on the 28th is followed by a fall in weight on the 30th. Taking next Chart V, which shows the same facts for subject No. 1, who took in comparison slight exercise (a moderate walk of 8 to 14 miles with no load, every day) it will be seen that the correspondence is much less exact. Thus, the fall in "water available" on the 19th is not shown in the weight until the 22nd, and the subsequent fall in the former factor on the 21st does not produce its full effect until the 26th. The rise of the 25th and 26th is shown in the weight of the 28th.

The first call on the "water available" is that for moisture needed to saturate the expired air. The amount of this will vary directly with the CO_2 in the expired air, and this again with the exertion. The variations from day to day will therefore correspond generally to those in the amount of water to be credited on account of hydrogen combustion, already referred to. Since these have been omitted from the table the relations between the variations in "water available" for perspiration and those in the weight may be considered to be fairly shown in Charts V to VII.

It would appear, therefore, that the demand for water for perspiration is not met immediately if there is a deficiency in that available, but is met more rapidly in the case of a man doing hard work than in one leading a more leisurely existence. In the

case of subject No. 2 (see Chart VI) who occupied an intermediate position as regards work, the correspondence between the two curves is more close than in the case of No. 1, less so than in No. 3.

It is interesting to note the extremely small amount of "water available" on certain occasions in the case of No. 1. In this case the amount of urine excreted not infrequently exceeded the amount of fluid actually drunk, though that was considerable. The connection between "water available" and water consumed is (as already stated) much less close in him than in the other two subjects, the presumption being that water ingested and not immediately required on account of work, is thrown out of the body through the kidneys at once.

It is also interesting to note that though the nitrogenous balance seems to point to the fact that protein tissue was being laid on and water being in this way fixed in the tissues, the amount of water available was diminishing. This is particularly noticeable in No. 2, where water available decreased almost as steadily as the nitrogen was accumulated.

The variations in the total amount of fluid consumed are interesting. At the outset the consumption of subject No. 3 was 3,960 c.c., and on the last day 2,180 c.c. In the severe march of 17·5 miles on the 19th the amount consumed was estimated at 4,060 grm., whilst on exactly the same march a week later it fell to 2,430. The relative humidity on the latter occasion approached saturation, whilst on the former it was about 62 per cent, probably the drier air of the earlier date, as well as want of condition, predisposed to greater apparent thirst. This diminution in the consumption of fluid was well marked also in the case of No. 2, who took part in almost all the marches, though without carrying any weight. For instance, taking the two dates already named, this subject consumed 1,800 c.c. of fluid on the first and only 1,200 c.c. on the second. That this decrease was probably almost entirely due to improved condition is shown by the fact that No. 1, who took comparatively little hard exercise, maintained a fairly steady consumption of fluid throughout.

Two practical points in connexion with march discipline seem deducible from these observations.

The first is that if a man has to go short of water for one day the effect on the water available for perspiration, that is, for temperature regulation, may persist, even in a well trained man for about forty-eight hours. The mere fact that a plentiful supply

of water is available on the next day will only tend to increase his urinary secretion, not to redress at once the disturbance in water content of his dehydrated tissues. A similar though perhaps a less marked effect will follow an uneven allowance of water on any one day, as, for instance, when water is not available on a long march, but only at its termination. It is extremely important, therefore, to regulate the supply not only from day to day, but also in the course of every day.

The second point is the importance of training in lessening the demand for "water available" due almost certainly to more efficient "condition." As long as a man is soft, therefore, his water supply needs far more careful regulation than when he has got into good compaigning condition. (It may be remarked that the fluid actually drunk did not include alcoholic stimulants. Water, only, either as such or in the form of tea, was consumed. Rum was issued on four occasions only, in the ordinary service allowance of $2\frac{1}{2}$ oz., on the same dates as it was received by the men.)

Much assistance and advice has been received from Dr. J. Haldane, F.R.S., not only in suggestions for the carrying out the work, but also in the compilation of the reports.

EXPLANATION OF TABLE HEADINGS.

(1) *Body-weight.*—Always weighed after breakfast and before defæcation.

(2) *Work in Calories* (*Subject* 3 *only*).—Work before the march mostly bicycling, except August 13, which represents fourteen mile walk.

(3) *Energy Value of Food.* (4) *Nitrogen in Food.*—Subjects No. 1 and 3 weighed their own food (cooked) for the days before the march, and, using Atwater's tables, the calorie value of and nitrogen in the food have been calculated. The food during the march was issued uncooked, and any unconsumed was weighed and allowed for.

(5) *Amount of Fluid Drunk.*—Both this and the food during the march represent the amount of food consumed and the amount of fluid taken between 7 p.m. and 7 p.m., thus, the energy value of the food shown against August 18 represents that consumed between 7 p.m. August 17 and 7 p.m. August 18.

(6) *Amount of Urine Passed.*—In the same way as in the case of food and drink this represents the amount of urine passed between 7 p.m. and 7 p.m. This rule only applies to the march, the estimations for food and fluid consumed, and urine passed, on the days previous to the march count from midnight to midnight.

(7) *Nitrogen in Urine.*—The twenty-four hour amount was mixed and a sample taken in a glass stoppered bottle; a crystal of thymol and a small quantity of chloroform were then added, and the bottles stoppered, tied, and waxed. On return to the College the total nitrogen was estimated by Kjeldahl's process.

(8) *Weight of Fæces.*—The total stool was collected in a glass preserved-fruit jar, with an air-tight glass stopper, about 20 c.c. H_2SO_4 were added and the whole well mixed with a small glass rod which was left in the jar. On return to the College these jars were opened and placed in a hot-air oven and evaporated to dryness, then cooled, weighed, the jar cleaned out, and weighed; the differences in these weights are the figures shown under the above heading. The whole mass was ground in a mortar, and 1 grm. taken for each determination of nitrogen by the Kjeldahl process.

0.004 grammes of nitrogen have been deducted for the paper used at each evacuation.

(9) *Nitrogen Balance.*—The amount of nitrogen in the stool of August 19 has been subtracted from the amount of nitrogen in the food of August 18, and the result has been compared with the nitrogen in the urine of August 18. On the days when there was no evacuation, half the amount of the nitrogen of the next stool has been deducted.

TABLE I.

SUBJECT No. 1.

Date	Body weight	Energy value of food	Nitrogen in food	Amount of fluid drunk	Amount of urine	Nitrogen in urine	Weight of fæces (dry)	Nitrogen in fæces	Nitrogen balance
	Kilos.		grm.	c.c.	c.c.	grm.	grm.	grm.	
Preliminary Period—									
August 7	..	2,053·3	11	1,160	1,650	11·55	..	1·50	− 2
,, 8	..	2,008·5	9	1,215	1,780	10·06	40	1·62	− 3
,, 9	..	..	..	..	..	..	52		
,, 11	..	1,936·3	12	975	2,850	11·09	..	1·73	
,, 12	..	..	..	..	2,160	10·04	39	..	− 1
March Period—									
,, 17	75·00	..	..	..	..	..	..	3·91	
,, 18	75·20	2,503	22·00	2,975	970	10·64	231	3·44	+ 8
,, 19	75·00	2,600	24·00	2,175	2,245	18·35	77	2·70	+ 3
,, 20	75·00	2,427	17·00	2,900	2,020	15·39	65	2·45	− 1
,, 21	75·40	3,244	20·00	2,125	2.425	18·06	50	4·75	− 3
,, 22	75·90	3,373	24·00	2,200	2,395	15·44	114	3·96	+ 5
,, 23	75·45	3,460	24·00	2,900	1,795	15·02	90	2·59	+ 6
,, 24	74·70	3,470	19·00	2,864	2,970	14·55	110	5·65	− 1
,, 25	74·65	3,355	23·00	2,850	2,400	11·89	165	2·16	+ 9
,, 26	73·75	3,355	23·00	2,900	2,055	13·52	65	2·38	+ 7
,, 27	73·50	3,553	23·00	2,800	2,010	13·22	92	·11	+10
,, 28	73·95	3,216	21·00	2,100	1,500	15·41	15	·84	+ 5
,, 29	73·90	3,833	23·00	2,200	1,670	15·43	33	..	+ 7
,, 30	73·90	..	..	..	..	..	*Nil*		

TABLE II.

SUBJECT No. 2.

Date	Body weight	Energy value of food	Nitrogen in food	Amount of fluid drunk	Amount of urine passed	Nitrogen in urine	Weight of fæces (dry)	Nitrogen in fæces	Nitrogen balance
	Kilos.		grm.	c.c.	c.c.	grm.	grm.	grm.	
March Period									
August 17	52·70	..	..	..	..	..	48	1·68	
,, 18	52·35	2,372	19	2,220	912	15·96	104	2·83	±
,, 19	52·30	2,437	26	1,800	910	18·11	55	1·25	+ 7
,, 20	52·80	2,260	16	2,100	1,270	17·60	85	1·77	− 3
,, 21	52·75	2,174	18	1,850	1,210	18·47	50	1·54	− 2
,, 22	52·70	3,637	24	1,640	920	13·39	*Nil*	*Nil*	+ 11
,, 23	52·45	3,324	26	1,635	875	13·30	50	1·62	+ 11
,, 24	52·80	3,714	20	1,500	840	12·32	54	1·84	+ 6
,, 25	52·60	3,780	25	1,350	990	14·02	55	1·78	+ 9
,, 26	53·50	2,943	24	1,200	1,105	14·85	7	1·14	+ 9
,, 27	52·20	3,685	22	1,300	910	12·51	85	2·78	+ 7
,, 28	52·60	3,089	23	1,350	870	15·27	48	1·58	+ 6
,, 29	52·55	3,615	24	1,200	920	13·13	13	1·57	+ 9
,, 30	52·45								

TABLE III.—Subject No. 3.

Date	Body weight (stripped)	Calorie value of food	Work in calories	Amount of fluid drunk	Amount of urine passed	Weight of faces (dry)	Nitrogen in food	Nitrogen in urine	Nitrogen in faces	Nitrogen balance	Carbo-hydrates in the food	Fat in the food	Protein in the food	Respective Proportions			Ether extract (faces)
														Protein	Fat	Carbo-hydrates	
	Kilos.			c.c.	c.c.	grm.	grm.	grm.	grm.		grm.	grm.	grm.				
Preliminary Period—																	
August 7	..	3,168	3,880	1,660	1,307	..	20	17·5	1·5	+ 1	330	159	122	1	1·00	3·00	
,, 8	..	2,853	3,667	1,772	1,057	62	24	17·3	1·2	+ 5	284	119	147	1	·75	2·00	
,, 9	71·84	2,526	3,593	1,188	1,220	58	20	18·4	1·6	± 0	265	110	126	1	1·00	2·00	
,, 10	72·10	2,757	4,110	1,460	1,350	59	22	20·0	4·7	− 2	381	107	136	1	1·00	3·00	
,, 11	72·10	2,609	3,675	1,410	1,460	103	21	20·8	1·2	− 1	369	128	131	1	1·00	3·00	
,, 12	..	2,828	3,350	1,222	1,198	59	19	19·2	2·0	− 2	308	135	130	1	1·00	2·00	
,, 13																	
,, 14	..	2,883	3,000	1,880	1,155		15	20·0	1·9	− 7	332	149	95	1	1·50	3·50	
,, 15	..	..	..	..	..	51											
,, 17	71·60																
										+ 6 − 12 − 6	324	129	127	1	1·00	2·50	
March Period—																	
August 18	70·95	2,276	4,433	3,110	960	46	17	19·1	1·5	− 4	337	87	107	1	·75	3·00	
,, 19	70·80	2,363	4,541	3,190	780	61	18	16·8	1·5	±	364	88	111	1	·75	3·00	
,, 20	71·00	2,011	4,262	2,240	890	30	14	19·3	2 2	− 7	314	46	88	1	·5	3·50	
,, 21	71·30	3,137	4,025	2,290	1,100	58	24	19·3	3·0	+ 2	408	173	147	1	1·00	2·00	
,, 22	71·15	3,843	4,631	2,470	1,115	98	25	15·3	2·8	+ 7	448	176	157	1	1·00	3·00	2·7
,, 23	70·90	3,763	3,000	1,250	1,130	90	27	16·1	1·9	+ 9	444	179	172	1	1·00	2·50	1·1
,, 24	71·50	3,952	4,001	1,640	1,170	63	22	20·8	·7	± 0	438	173	136	1	1·25	3·00	
,, 25	70·45	3,526	4,433	2,160	1,000	31	24	15·2	1·9	+ 7	412	171	148	1	1·00	3·00	0·17
,, 26	70·20	3,720	4,541	1,580	885	57	25	15·7	*Nil*	+ 9	441	172	156	1	1·00	3·00	
,, 27	69·65	3,697	4,262	2,155	830	*Nil*	23	15·5	1·1	+ 6	412	171	141	1	1·00	3·00	
,, 28	70·40	3,865	3,000	2,410	1,780	40	26	19·2	3·5	+ 3	482	176	161	1	1·00	3·00	
,, 29	70·75	3,967	4,625	1,500	1,340	70	26	19·3	*Nil*	+ 7	460	176	166	1	1·00	3·00	
,, 30	69·70	..	..	..	..	*Nil*											
Totals ..		40,120	49,754							− 11 + 50							
Averages..		3,343	4,146							+ 39	413	149	140	1	1·00		