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NATIONAL HEALTH INSURANCE

MEDICAL RESEARCH COMMITTEE

An Investigation of the Flexner-Y Group of Dysentery Bacilli

Approved for publication by the Medical Research Committee, October 18, 1918.

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(National Health Insurance.)

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15 BUCKINGHAM STREET,
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INTRODUCTION

THE following Report upon the Flexner-Y group of dysentery bacilli gives the results of long and patient work by the late Dr. H. S. Gettings so far as it has been found possible to retrieve them from his laboratory note-books and other papers as these were found after his sudden and untimely death, on June 15, 1918. The work of arranging them for the press has been kindly undertaken for the Committee by Captain S. R. Douglas.

The circumstances in which these investigations were begun and continued are mentioned by Captain Douglas in his introduction to the report. In spite of an inherited physical infirmity, Dr. Gettings gave to his work a prolonged patience and skill that won the respect of all who knew him. The lines of inquiry he followed were leading to many points of scientific interest and they gave promise of practical gains in the improved control of disease. It is part of the tragedy of his untimely death last June that he should thus have failed to reap the harvest of these preliminary studies himself and to bring his results into application to the object he had kept so long in view—the prevention and control of dysentery in asylums and other communities. It is further to be deplored that it did not fall to him even to present his own account of the work he had completed at the time of the accident which led to his death. In great part his notes were in such order that his findings could be given with confidence by one who, like Captain Douglas, had worked side by side with him and knew the details of his methods; but we have lost much, no doubt, that Dr. Gettings had in mind at the time for publication.

The material he left shows clearly, however, that after trial and rejection of cultural and of ordinary agglutination methods for distinguishing clearly between different sub-groups of Flexner dysentery bacilli, he had by means of tests based on the absorption of agglutinins obtained sharp discrimination between four sub-groups, of which one appeared to be identical with the so-called Y group previously described. Dr. Gettings's clear recognition of these four sub-groups, which he had discussed freely with other

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workers, has already given aid to the recognition of organisms of the Flexner group whose characters had been found elsewhere to be 'aberrant' or which had missed detection. Further work by others is already being applied to bringing knowledge of these sub-groups into use for diagnostic purposes and for preventive work.

The Committee are greatly indebted to Captain Douglas for the pains he has given to the preparation of Dr. Gettings's notes for this report. All who know the relation of laboratory records to a finished paper will appreciate at once both what we may have lost in losing Dr. Gettings's own presentation of his results, and what is owed to Captain Douglas for his care in doing this office for his fellow worker.

MEDICAL RESEARCH COMMITTEE,
15 Buckingham Street,
Strand, W.C. 2.

January 30, 1919.

AN INVESTIGATION OF THE FLEXNER-Y GROUP OF DYSENTERY BACILLI

ISOLATED FROM CASES OF ASYLUM DYSENTERY OCCURRING AT WAKEFIELD ASYLUM, WITH SPECIAL REFERENCE TO THE POSSIBILITY OF SUB-DIVIDING THIS GROUP OF BACILLI.

By the late H. S. Gettings, L.R.C.P., L.R.C.S. Edin., D.P.H.

Arranged for the press by S. R. Douglas, M.R.C.S., L.R.C.P. (Capt. I.M.S., retd.)

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I. Introduction.

During the period (1912–16) in which he was Pathologist at the Wakefield Asylum Dr. H. S. Gettings made an extended study of asylum dysentery. Some of the results were embodied in the following papers :

(1) Dysentery. Past and Present. *Journal of Mental Science*, 1913, October.

(2) The Detection of a Dysentery 'Carrier'. *Journal of Mental Science*, 1914, October.

(3) Bacillary Dysentery. *Trans. Soc. of Tropical Med. and Hyg.*, 1915, Vol. VIII, No. 4.

In the first paper, which is one of great interest, he was able to show from an examination of the historical records of the Wakefield Asylum that dysentery had never been absent from that institution for any long period since its foundation. Reasoning from these and other facts found in the records, he came to the conclusion that the disease was probably kept 'alive' by the agency of 'carriers'.

In the second paper, which was published a year later, he was able to confirm this opinion from the study of a small outbreak of dysentery which was traced to a 'carrier'. This 'carrier' had most probably been the cause of other small outbreaks.

During the time he was occupied in investigating the cases of dysentery he collected a large number of strains of the Flexner-Y group of dysentery bacilli. While still acting as Pathologist of the Asylum he began to examine the various strains of this group of bacilli, his object being to ascertain whether they all fell into a single group or could be divided into a number of sub-groups.

In view of the importance of this work, in relation not only to the problems of asylum dysentery but also to the study of dysentery among our Forces overseas, the Medical Research Committee in 1915 invited Dr. Gettings to give his whole time, on their behalf, to the continuation of his inquiries. Early in 1916 they were able to obtain laboratory accommodation for him by the courtesy of the Inoculation Department, St. Mary's Hospital, and here his work progressed so well that, in spite of interruptions due to some other inquiries of more urgent importance which he undertook and completed,[1] it had reached its final stage at the beginning of 1918.

From February of this year, in consequence of an injury received in a railway accident, Dr. Gettings was laid up for some months. By June he had, however, seemingly almost completely recovered when he suddenly became seriously ill, and greatly to the regret of all his associates, this illness proved fatal on June 15, 1918.

In order that the record of this work—the result of many years careful and accurate experimentation—should not be lost, the Medical Research Committee, with the consent of his relatives, requested me to examine Dr. Gettings's papers and, if possible, to write a report on the work so far as it was completed. It was with considerable trepidation that I consented to attempt this task, knowing the difficulty of following the notes of another worker. However, on examination, Dr. Gettings's records were found to be so fully and clearly stated that I have been able to follow the details of the various experiments. In addition I had the further advantage of having, in the past, often discussed the work with him. I hope that the following report may give his more important results. If this aim is attained I shall be content, as it will indicate that many difficulties, at first seemingly unsurpassable, have been overcome. It is, however, quite impossible to hope that all the points will be given, or that this report will compare in value with that which Dr. Gettings would himself have compiled, as probably

[1] The result of this work appeared in No. 15, Special Report Series of the Medical Research Committee, 1918.

many of his ideas and facts were never placed on record in his note-books.

II. Object of the Inquiry.

The object of the work was to ascertain if the Flexner-Y group of dysentery bacilli could be divided into a number of sub-groups, and Dr. Gettings evidently asked himself the following questions:

I. Is it feasible to do this by the study of the biochemical reactions of the bacilli?

II. Is it feasible to do this by the study of either of the following reactions of immune sera?

(*a*) The agglutination of emulsions of the bacilli by a serum made by inoculating animals with a vaccine prepared from a single strain.

(*b*) The absorption by the various bacilli of the specific agglutinins from a serum prepared by means of a single strain of the bacilli.

III. Biochemical Reactions.

Is it possible to divide the members of the Flexner-Y group of bacilli into a number of sub-groups by the study of their biochemical reactions?

Technique, &c.

Plates were made from the emulsified faeces on MacConkey's bile salt lactose neutral red agar, and after incubation a number of the white colonies were picked off and pure cultures made. These were then planted in lactose broth, litmus being used as the indicator, and the broth being made with the meat extract and peptone.

If the bacillus was found not to ferment lactose after incubation for at least seven days, the following tests were made.

The bacillus was planted in broths containing (i) glucose, (ii) mannite, (iii) dulcite, (iv) saccharose, (v) salicin. These were incubated for at least seven days and examined daily for any fermentation reactions. Cultures were also made into litmus milk and into peptone water; the milk culture was incubated for fourteen days and examined at intervals for the presence of acidity or any other change of reaction and also for clotting; the peptone water culture was incubated for seven days and then tested for the presence of indol.[1]

It will be seen that the scheme of these tests is that recommended by C. J. Lewis, *L. G. B. Report*, 1910–11. These tests having been completed, any strains that produced acid in glucose and mannite

[1] The exact method used in this test I have been unable to ascertain. S. R. D.

broths but failed to ferment dulcite, saccharose, and salicin were provisionally classed as members of the Flexner-Y group. The results of the other two tests, as will be shown later, were not quite constant, but usually indol was present in the peptone water culture and the milk culture remained alkaline. The provisional diagnosis was confirmed by finding that emulsions, made usually from young agar cultures, were agglutinated by a polyvalent agglutinating serum for the Flexner-Y group.

In attempting to split the various strains, which had been collected, into sub-groups by means of biochemical reactions Lewis's 'secondary series' of sugars were employed. These consist of (i) sorbite, (ii) rhamnose, (iii) maltose, (iv) levulose, (v) galactose, (vi) mannose, (vii) raffinose, (viii) dextrin, (ix) inulin, (x) adonite, (xi) glycerin.

Using these tests, the results obtained by investigating 285 strains of the Flexner group isolated from cases were recorded.

On examining these results it is at once obvious that it is quite impossible to divide this group of bacilli into sub-groups by such reactions, and in the following five tables sufficient results are given to show the uselessness of this method.

In Table I, the results are given of the fermentation reactions, &c., of thirty-six strains each isolated from a single case; those strains being selected which were afterwards used in the second portion of this research in carrying out agglutination and absorption tests.

The following signs and abbreviations are used in these tables:

A indicates that the carbohydrate in question, or milk culture, was fermented, acid being formed.

— indicates that the carbohydrate was unaffected or that the indol test was negative.

+ indicates that the indol test was positive.

Alk indicates that the milk culture remained alkaline, the alkalinity being increased if any change occurred.

A & C indicates that acid and clot were produced in the milk culture.

Dcl indicates that there was decolorization of the litmus used as an indicator.

s following A indicates slight production of acid.

TABLE I

No. of Case	No. of Strain	Primary Series: Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Secondary Series: Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
1	5	A	A	—	—	—	—	A	—	—	—	A	A	A	A.s	—	A.s	—	—
6	1	A	A	—	—	—	—	A	—	—	A.s	A	A	A	A	A.s	—	—	—
7	1	A	A	—	—	—	—	A	—	—	A	A	A	A	A	—	—	—	—
8	1	A	A	—	—	—	—	A	—	—	A	A	A	A	A	A	—	—	—
9	1	A	A	—	—	—	+	Alk	—	—	A	A	A	A	A	A	—	—	—
10	1	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	A	—
11	1	A	A	—	—	—	+	Alk	—	—	—	A	A	—	A	—	—	A	—
17	1	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
19	1	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	—	—	—	—
22	1	A	A	—	—	—	—	Alk	—	—	A	A	A	A	—	—	—	—	—
23	1	A	A	—	—	A	—	A & C	—	—	—	A	A	A	A	—	—	—	—
24	1	A	A	—	—	—	+	Alk	—	—	A	A	A	A	A	Del	—	—	—
26	1	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
28	1	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
38	1	A	A	—	—	—	+	A	—	—	—	A	A	A	—	—	—	A	—
39	1	A	A	—	—	—	+	A	—	—	A	A	A	—	—	—	—	A	—
40	1	A	A	—	—	—	+	A	A	—	A	A	A	—	—	—	—	A	—
47	1	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
48	1	A	A	—	—	—	+	Alk	A	—	A	A	A	—	—	Del	—	—	—
49	1	A	A	—	—	—	—	Alk	—	—	—	A	A	—	—	—	—	—	A
51	1	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
55	1	A	A	—	—	—	+	Alk	A	A	A	A	A	A	—	—	—	—	—
56	1	A	A	—	—	—	—	A & C	—	—	A	A	A	A	A	—	—	—	—
64	1	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	—	—
65	1	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
67	1	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
68	4	A	A	—	—	—	+	Alk	A	—	A	A	A	A	A	—	—	—	—
69	1	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
70	1	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
74	6	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
75	9	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
76	1	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	—	—
78	7	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
79	2	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
86	1	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
91	1	A	A	—	—	—	+	A	—	—	—	A	A	—	—	—	—	—	—

Even in the primary series of tests, those dealing with the production of indol and the action of the bacilli on milk show very considerable discrepancies.

Unfortunately, as mentioned above, the exact method used for testing for indol is unknown, and some of the variations may be due to impurities of chemicals or media, &c., or to the method employed. As regards milk cultures it is well recognized that if such an unstable body is used as a culture medium aberrant results will occur.

When the results obtained by using the so-called secondary series of tests are examined, it is very clearly demonstrated that biochemical reactions are without any practical use in arranging bacilli in subgroups.

In Tables II, III, IV, and V the results of the primary and secondary tests obtained with a series of Flexner-Y bacilli, isolated from one particular case on a certain date, are given, and it is at once clear that even these results show many remarkable differences.

TABLE II

No. of Case	No. of Strain	Primary Series: Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Secondary Series: Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
10	1	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	A	—
	2	A	A	—	—	—	—	Alk.AC	—	—	—	A	A	—	A	—	—	A	—
	3	A	A	—	—	—	+	AC	—	—	A	A	A	—	A	—	—	A	—
	4	A	A	—	—	—	—	Alk.AC	—	—	—	A	A	—	A	—	—	A	—
	5	A	A	—	—	—	+	Alk	—	—	A	A	A	—	A	—	—	A	—
	6	A	A	—	—	—	+	Alk.AC	—	—	—	A	A	—	A	—	—	A	—
	7	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	A	—	—	—
	8	A	A	—	—	—	+	Alk.AC	—	—	A	A	A	—	A	—	—	A	—

TABLE III

No. of Case	No. of Strain	Primary Series: Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Secondary Series: Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
55	1	A	A	—	—	—	+	Alk	A	A	A	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	3	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	4	A	A	—	—	—	+	Alk	A	A	A	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	+	Alk	A	A	A	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	7	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	8	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	—	—
	9	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	—	—	—	—

TABLE IV

No. of Case	No. of Strain	Primary Series: Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Secondary Series: Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
65	1	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	+	Alk	A	A	A	A	A	A	—	A	—	—	—
	6	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	Del	—	—	A
	7	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	8	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—

TABLE V

No. of Case	No. of Strain	Primary Series: Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Secondary Series: Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
68	4	A	A	—	—	—	+	Alk	A	—	A	A	A	A	A	—	—	—	—
	5	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—

In a former paper [1] Dr. Gettings pointed out that these variations of biochemical reactions were probably due to a different physiological action of the actual bacilli. The carbohydrate which was specially selected for this investigation was maltose, owing to its having been frequently stated that the true Flexner group could be separated from the Y group of bacilli by their respective actions on this sugar. The above conclusion was arrived at after an extended series of experiments, in which pure cultures of some of the strains were planted out on to plates of MacConkey's neutral red bile salt agar, to which maltose had been added in place of the lactose. Such a plate yielded colonies of two types, one of which was coloured red owing to its power of fermenting maltose; the other remained colourless, as the bacilli which gave rise to them were unable to ferment this sugar. Sub-cultures made from such colonies preserved these reactions, at any rate, for a time.

The full details of the biochemical reactions of the 285 strains isolated from 39 cases are given in Appendix I.

IV. Reactions with Specific Immune Sera.

1. *Is it possible to divide the members of the Flexner-Y group of dysentery bacilli into a number of sub-groups by means of the agglutination of emulsions of the different strains of bacilli by sera prepared through the medium of a single strain?*

Two specific agglutinating sera were prepared by inoculating rabbits with a series of doses of a vaccine prepared in the one case from the bacillus, Case 10, Strain 1, and in the other, Case 86, Strain 1—the first of these doses was given subcutaneously but the later ones intravenously.

The titre of these sera seemed to have varied considerably at different dates, and in the following table some of the figures found in the note-books are given. Although it cannot be stated absolutely, it is probable that fresh sera were prepared with both strains about May and June 1917.

TABLE VI

Serum prepared from Case 10, Strain 1		Serum prepared from Case 86, Strain 1	
Date	Titre	Date	Titre
9.6.14	3200 +	9.6.14	1600 + 3200 P
10.7.14	6400 +	9.7.14	6400 +
18.8.15	1600 + 3200 P	18.8.15	3200 + 6400 P
*14.6.17	4000 +	22.9.15	800 + 1600 P
30.6.17	1000 + 2000—	2.10.15	1000 + 3200 P
25.7.17	1000 + 2000—	9.10.15	800 + 1600 P
		*14.6.17	2000 + 4000 P
		30.6.17	1000 + 2000—

* At this date most probably freshly prepared sera were employed.

In this table and also in the following one + indicates complete agglutination; —, the absence of agglutination, P, partial agglutination, T. S., type strain.

[1] *Transact. Trop. Soc. Med. and Hyg.*, 1915, vol. vi.

TABLE

BACILLUS USED IN THE PREPARATION OF THE SERUM. CASE 10, STRAIN 1

Date of Experiment	9.6.14		10.7.14					2.10.15		20.10.15		9.10.15					20.6.17		Case and Strain with which agreement is shown by absorption test	
Dilution of Serum	1 in 400	1 in 800	1 in 400	1 in 800	1 in 1600	1 in 3200	1 in 6400	1 in 400	1 in 800	1 in 400	1 in 800	1 in 50	1 in 100	1 in 200	1 in 400	1 in 800	1 in 500	1 in 1000		
No. of Case and Strain tested																				
Case 10 Strain 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	T.	S.
1 5	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
6 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
7 1	+	+	...	...	...	...	...	+	+	+	+	...	...	...	...	...	...	...	10	1
8 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
9 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
11 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
17 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	..	...	...	10	1
19 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
22 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
23 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
24 1	+	+	+	+	+	+	+	—	—	+	+	...	...	...	...	...	...	...	10	1
26 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
28 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
38 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	..	...	...	10	1
39 1	...	..	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
40 1	+	+	+	+	P	—	—	—	—	—	—	+	+	+	+	+	—	—	170	1
47 1	—	—	...	...	...	...	...	—	—	+	+	+	+	+	+	+	...	...	—	—
48 1	...	...	...	...	...	...	...	—	—	—	—	+	+	+	P	—	...	...	86	1
49 1	+	+	+	+	+	+	+	..	...	...	...	...	...	...	...	...	...	...	10	1
50 1	+	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..	...	10	1
51 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10	1
55 1	...	...	...	...	...	...	...	—	—	—	—	+	+	P	—	—	...	...	86	1
56 1	+	—	..	...	...	...	...	+	+	+	+	+	+	+	+	+	...	...	10	1
64 1	...	..	...	...	...	...	...	—	—	+	+	+	+	+	—	—	...	...	86	1
65 1	...	...	...	...	...	...	...	—	—	+	+	...	...	...	...	..	...	...	86	1
67 1	...	...	...	...	...	...	...	—	—	...	...	...	...	...	...	...	...	...	10	1
68 1	...	..	...	...	...	...	...	—	—	...	...	...	...	...	...	...	...	...	86	1
69 1	...	...	...	...	...	...	...	—	—	...	...	...	...	...	...	...	...	...	86	1

Throughout these experiments the following technique was employed:

The emulsions were made by suspending in 0·85 per cent. salt solution enough bacilli from an agar culture of eighteen to twenty-four hours to make a conveniently thin emulsion.

Usually only two dilutions of serum were employed, these were made in bulk, and in some of the experiments were considerably below the full titre of the serum.

The tests were carried out in small test-tubes ($4 \times \frac{3}{8}$ inch), and equal volumes of the emulsions and dilutions of serum were mixed in these. When a series of tubes had been filled in they were placed in a water-bath. In the earlier experiments a temperature of 37° C. was employed, while later 45° C. was at times used. Readings were made after the tubes had been in the water-bath from two to four hours; after this reading the tubes were removed from the water-bath and kept on the bench until the following morning, when a confirmatory reading was made.

VII

BACILLUS USED IN THE PREPARATION OF THE SERUM. CASE 86, STRAIN 1

Date of Experiment	10.6.14		10.7.14					28.10.14		6.9.15		9.10 15					20.6.17	
Dilution of Serum	1 in 400	1 in 800	1 in 400	1 in 800	1 in 1600	1 in 3200	1 in 6400	1 in 400	1 in 800	1 in 400	1 in 800	1 in 50	1 in 100	1 in 200	1 in 400	1 in 800	1 in 500	1 in 1000
No. of Case and Strain tested																		
Case 10 Strain 1	—	—	...	...	...	...	...	+	+	—	—							
1 5	—	—	...	...	...	...	...	+	+	—	—							
6 1	—	—	...	...	...	...	...	+	—	—	—							
7 1	—	—	...	...	...	...	...	+	+	—	—							
8 1	—	—	...	...	...	...	...	+	+	—	—							
9 1	—	—	...	...	...	...	...	+	+	P	P							
11 1	—	—	...	...	...	...	...	+	+	P	P							
17 1	—	—	...	...	...	...	...	+	+	—	—							
19 1	—	—	...	...	...	...	...	+	+	P	P							
22 1	—	—	...	...	...	...	...	+	+	—	—							
23 1	—	—	...	...	...	...	...	+	+	—	—							
24 1	—	—	...	...	...	...	...	+	+	—	—							
26 1	—	—	...	...	...	...	...	+	+	—	—							
28 1	—	—	...	...	...	...	...	+	+	—	—							
38 1	—	—	...	...	...	...	...	+	+	P	P							
39 1	—	—	...	...	...	...	...	+	+	—	—							
40 1	—	—	...	...	...	...	...	—	—	—	—	...	...	...	...	...	—	—
47 1	—	—	...	...	...	...	...	+	+	P	P	+	+	P	—	—		
48 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
49 1	—	—	...	...	...	...	...	+	+	P	P							
50 1	—	—	...	...	...	...	...	+	+	—	—							
51 1	—	—	...	...	...	...	...	+	+	P	P							
55 1	+	+	+	+	+	+	+	+	+	+	+							
56 1	—	—	...	...	...	...	...	+	+	—	—	+	P	—	—	—		
64 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
65 1	+	+	+	+	+	+	+	+	+	+	+							
67 1	+	+	+	+	+	+	+	+	+									
68 1	+	+	+	+	+	+	+	+	+	+	+							
69 1	+	+	+	+	+	+	+	+	+	+	+							

Salt solution controls were employed with every emulsion, and frequently, when discordant results were obtained, a series of tubes, in which were placed dilutions of normal rabbit's serum, were employed as a control.

In the following table the results obtained with the strains selected from the first twenty-nine cases are given; these are sufficient to show how much overlapping there was in the results.

A full record of the results of the agglutination tests obtained with two sera mentioned above and a selected strain from ninety-nine different cases is given in Appendix II. To these have been added, whenever the data could be given, the results obtained by means of absorption tests which are described in the last portion of the paper.

To sum up the conclusion drawn from these experiments, it may be stated that although these results were much more encouraging than those obtained from the study of the biochemical reactions they were not sufficiently sharp or constant to enable definite sub-

groups to be made. However, they strongly supported the supposition that the Flexner-Y group of dysentery bacilli did consist of a number of sub-groups which were very nearly related to one another. It may here be noted that on several occasions various sera, including both those prepared by means of a single strain, and others prepared by means of several strains of this group of bacilli, were tested to ascertain if they had any power to agglutinate bacilli not belonging to this group such as *B. typhosus* and *B. dysenteriae*, Shiga; these experiments gave uniformly negative results.

2. *Is it possible to divide the members of the Flexner-Y group of bacilli into a number of sub-groups by means of the absorption of the specific 'agglutinins' by the different strains of bacilli from sera prepared through the medium of a single strain?*

The technique employed in carrying out the absorption tests was as follows: the bacillus to be tested was planted out on agar slopes which were then incubated for eighteen to twenty-four hours at 37° C. After removing any water of condensation that might have collected, the growth was washed off the slope with a minimum of salt solution, usually about 1 c.c.; if this emulsion was not thick enough, it was added to another agar culture and the growth from this second tube was then washed off with it. When a sufficiently thick emulsion had been prepared, a measured quantity was added to a measured quantity of the serum; usually 1½ to 2 c.c. of the emulsion were added to 1½ to 2 c.c. of a 1 in 10 dilution of the serum. This mixture was made in a small test-tube which was placed either in a water-bath or the incubator at 37° C. for from two to four hours, and then kept at laboratory temperature overnight.

It was found out by experiment that in order to remove all the agglutinins from a 1 in 20 dilution of one of the immune sera employed in these tests, it was necessary to add the growth from two agar slopes. With smaller quantities, for instance, the growth from one agar slope, the agglutinins were only partially removed. This serum had an agglutination titre of 1 in 1500. In order to avoid errors, throughout the tests, sufficient bacilli were always added to remove *all* the agglutinins from the dilution of the serum employed.

In the following table the degree of agglutinating power remaining after a 1 in 20 dilution of a serum had been treated with varying quantities of the homologous bacillus is shown.

TABLE VIII

Serum tested	Dilutions of Serum							
	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640	1 in 1280	1 in 1500	1 in 2000
Untreated	+	+	+	+	+	+	+	—
Absorbed with one loopful of bacilli	+	+	+	+	P	—	—	—
Absorbed with one loopful of bacilli	+	+	+	+	—	—	—	—
Absorbed with two loopfuls of bacilli	+	+	+	P	—	—	—	—
Absorbed with three loopfuls of bacilli	+	+	+	P	—	—	—	—
Absorbed with bacilli from ½ an agar slope	+	+	P	—	—	—	—	—
Absorbed with bacilli from 1 agar slope	+	+	—	—	—	—	—	—
Absorbed with bacilli from 2 agar slopes	—	—	—	—	—	—	—	—

The sign + indicates that when the serum either had been absorbed with the quantity of bacilli mentioned or had not been treated, agglutination of an ordinary thin emulsion took place in that dilution.
P indicates partial agglutination.
— indicates that no agglutination took place.

The next day the tubes containing the mixtures of thick emulsion and serum were centrifuged until the supernatant fluid was quite clear; this was pipetted off, and dilutions such as 1 in 40, 1 in 80, 1 in 160, &c., having been made, two series of tubes were filled in with equal quantities of these dilutions and thin emulsions of (i) the bacillus used in absorbing the serum, (ii) the bacillus used in the preparation of the serum. If the serum was found to have lost all power of agglutinating both the bacillus used in absorbing the serum and also the bacillus used in the preparation of the serum, the two bacilli belonged to the same sub-group; if, however, it was found that the serum had the power of agglutinating the bacillus used in the preparation of the serum, although it had completely lost the power to agglutinate emulsions of the bacillus used in absorbing the serum, it belonged to another sub-group.

Using this technique a number of strains of the Flexner-Y group were tested, one strain from each case being investigated. As the strains were placed in different sub-groups it was found that there were some which could not be allocated by means of the sera available; it was therefore necessary to prepare other sera, and in all five sera were employed, with the result that all the strains, 87 in number, were found to correspond with one or other of the strains used in preparing these five sera, with the exception of three. The results obtained by this technique were perfectly sharply defined, and no matter how often the experiments were repeated the results were always exactly the same.

Leaving out one small group consisting of only two strains, which will be discussed later, four definite sub-groups of the Flexner-Y group of bacilli were identified.

Sub-group 1, in which Case 10, Strain 1, was used in preparing the serum, consisted, in all, of forty members.

Sub-group 2, in which Case 86, Strain 1, was used in preparing the serum, consisted, in all, of thirty members.

Sub-group 3, in which Case 170, Strain 1, was used in preparing the serum, consisted, in all, of eleven members.

Sub-group 4, in which Case 311, Strain 1, was used in preparing the serum, consisted, in all, of eleven members.

In other words, of the 92 strains tested, 43·5 per cent. belonged to Sub-group 1, 32·6 belonged to Sub-group 2, 12 per cent. to Sub-group 3, and 12 per cent. to Sub-group 4. The three remaining strains could not be placed in any of these sub-groups.

In the following tables are shown the sub-groups to which each strain belongs as far as they have been worked out. These are also given in Table VII, in the text, and Tables 1, 2, 3, 4, and 5, Appendix II.

TABLE IX

No. of Case and Strain showing agreement with Case 10, Strain 1, by absorption test

Case	Strain	Case	Strain	Case	Strain	Case	Strain	Case	Strain
1	5	22	1	50	?	130	1	307	2
6	1	23	1	51	1	131	1	318	1
7	1	24	1	56	1	136	1	319	1
8	1	26	1	67	1	139	1	321	1
9	1	28	1	91	1	154	1	322	1
11	1	38	1	92	10	168	1	325	1
17	1	39	1	100	1	169	1	336	?
19	1	49	1	113	1	269	1		

TABLE X

No. of Case and Strain showing agreement with Case 86, Strain 1, by absorption test

Case	Strain	Case	Strain	Case	Strain	Case	Strain	Case	Strain
48	1	70	1	126	1	149	1	221	2
55	1	74	6	128	1	176	1	222	2
64	1	75	9	137	1	178	1	271	5
65	1	76	1	141	1	185	1	291	1
68	4	78	7	144	1	189	1	326	2
69	1	79	2	148	1	196	1		

TABLE XI

No. of Case and Strain showing agreement with Case 170, Strain 1, by absorption test

Case	Strain	Case	Strain	Case	Strain	Case	Strain	Case	Strain
40	1	156	1	159	3	164	1	167	1
79a	4	157	1	161	1	165	1	184	1

TABLE XII

No. of Case and Strain showing agreement with Case 311, Strain 1, by absorption test

Case	Strain	Case	Strain	Case	Strain	Case	Strain	Case	Strain
126*a*	?	200	1	225	1	260	3	270	1
172	1	219	2	231	?	268	1	290	1

As regards Case 275, Strain 2, one strain, namely Case 252, Strain 1, was found to be in complete agreement with it by the absorption test. On investigating these cultures further the bacilli were found to be motile, no indol was produced by either culture, and their growth in litmus milk produced no change in that medium. Emulsions of Case 275, Strain 2, were on several occasions put up with dilutions of an antityphoid agglutinating serum obtained from the Lister Institute, but no definite agglutination was ever recorded even when comparatively low dilutions were employed. A serum, however, prepared by inoculating a rabbit with a vaccine prepared from this bacillus (Case 275, Strain 2) agglutinated emulsions of stock typhoid cultures, and of the bacillus in question, to exactly the same titre; further absorption tests both with the Lister Institute typhoid agglutinating serum and the serum prepared by means of the bacillus Case 275, Strain 2, demonstrated that this bacillus and also that isolated from Case 252, Strain 1, were undoubted typical *B. typhosus*, which were evidently unaffected as regards agglutination by the high titre (1 in 6,000) typhoid agglutinating serum obtained from the Lister Institute.

From agglutination tests the sub-group agreeing with Case 170, Strain 1, appears to be identical with the 'Oxford'-Y strain, but this cannot be stated definitely as the agglutination tests were not performed with full-titre dilutions of the serum, and no record of any absorption tests can be found.

V. Summary and Conclusions

(1) From the first part of the paper, in which the biochemical reactions are studied, the following conclusions may be drawn; that, although the biochemical reactions with a certain small selected number of carbohydrates are, as is usually recognized, extremely useful as a *rough* means of identification of members of the coliform group of bacilli, it is quite futile to attempt to classify a group of very nearly related bacilli, such as the Flexner-Y group of dysentery bacilli, into definite sub-groups by using an extended series of these carbohydrates such as the 'secondary series' introduced by Lewis.

Even the use of maltose as a means for distinguishing the Y type of dysentery bacilli from the typical Flexner type was shown to give fallacious results.

(2) In the second portion of the paper dealing with ordinary agglutination tests it was shown, without in any way detracting from the value of such tests for definitely recognizing the various

bacteria of the coliform group of bacilli, that it was not feasible to divide by such means the Flexner-Y group of dysentery bacilli into a number of sharply defined sub-groups, the results obtained being neither sufficiently sharp nor constant. These tests, however, when they were carried out with sera prepared through the medium of a single strain, strongly confirmed the supposition that the Flexner-Y group of dysentery bacilli did consist of a number of sub-groups, the members of which were very nearly related to one another.

(3) In the last portion of the paper, which was unfortunately not quite, though very nearly, completed, the tests in which the different strains of the Flexner-Y group were identified by means of the absorption of the agglutinins from specific sera gave both very sharp and very constant results, and by the data thus arrived at it was possible to place 92 out of the 95 strains of the Flexner-Y group of dysentery bacilli that were investigated into four sub-groups.

Sub-group 1 consisted of forty members, that is 43·5 per cent.
Sub-group 2 consisted of thirty members, that is 32·6 per cent.
Sub-group 3 consisted of eleven members, that is 12 per cent.
Sub-group 4 consisted of eleven members, that is 12 per cent.

The remaining three strains could not be placed in any of the above, so that there is at least one other sub-group unidentified.

Certain evidence which, however, is not conclusive points to Sub-group 3 being identical with that usually called the Y group of dysentery bacilli.

APPENDIX I

In Appendix I the details of the biochemical reactions of 285 strains of the Flexner-Y group of bacilli isolated from thirty-nine cases are recorded.

Throughout these tables the following symbols and abbreviations are employed :

A indicates the fermentation of a certain carbohydrate with the production of acid, or that acid was produced in a milk culture.

A.s and A.v.s indicates that the production of acid was slight or very slight.

+ indicates that indol was produced after the bacillus had been growing in peptone water for seven days.

— indicates that no fermentation of the carbohydrate in question occurred, or that no indol was produced in a peptone water culture.

Alk indicates that the milk culture was rendered more alkaline.

A & C indicates that acid and clot were both produced in the milk culture.

Dcl indicates that the litmus used as an indicator was decolorized.

DETAILS OF BIOCHEMICAL REACTIONS OF VARIOUS CASES

		Primary Series							Secondary Series										
No. of Case	No. of Strain	Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
1	2	A	A	—	—	—	+	A	—	—	—	A	A	A	A	—	—	—	—
	3	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	4	A	A	—	—	—	—	A	—	—	—	A	A	A	A.s	—	A.s	—	—
	5	A	A	—	—	—	—	A	—	—	—	A	A	A	A.s	—	—	—	—
	6	A	A	—	—	—	—	A	—	—	—	A	A	A	A.s	—	—	—	—
	7	A	A	—	—	—	—	A	—	—	A.s	A	A	A	A	—	—	—	—
6		A	A	—	—	—	—	A	—	—	A.s	A	A	A	A	A.s	—	—	—
	2	A	A	—	—	—	—	A	A.s	—	A	A	A	A	A	A	A	—	—
	3	A	A	—	—	—	—	A	—	—	A.s	A	A	A	A	A.s	A.s	—	—
	4	A	A	—	—	—	—	A	A	—	A	A	A	A	A	A.s	A.s	—	—
	5	A	A	—	—	—	—	A	—	—	A	A	A	A	A	A.s	A.s	—	—
	6	A	A	—	—	—	—	A	—	—	A.s	A	A	A	A	A.s	A.s	—	—
	7	A	A	—	—	—	—	A	—	—	A.s	A	A	A	A	A	A.s	—	A
	8	A	A	—	—	—	—	A	A.s	—	A	A	A	A	A	A	A.s	—	—
	9	A	A	—	—	—	—	A	—	—	A	A	A	A	A	A	A.s	—	—
	10	A	A	—	—	—	—	A	—	—	A	A	A	A	A	A	A.s	—	—
7	1	A	A	—	—	—	—	A	—	—	A	A	A	A	A	—	—	—	—
	2	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	3	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	4	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	5	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	6	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	7	A	A	—	—	—	—	A	A	—	A	A	A	A	A	A	—	A	—
	8	A	A	—	—	—	+	A	—	—	A	A	A	A	A	—	—	—	—
	9	A	A	—	A.v.s	—	—	A	—	—	A	A	A	A	A	—	—	—	—
	10	A	A	—	—	—	—	A	—	—	A	A	A	A	A	—	—	—	—

		Primary Series							Secondary Series										
No. of Case	No. of Strain	Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
8	1	A	A	—	—	—	—	A	—	—	A	A	A	A	A	A	—	—	—
	2	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
9	1	A	A	—	—	—	+	Alk	—	—	A	A	A	A	A	A	—	—	—
	2	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	A	—	—	—
	3	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	A	—	—	—
	4	A	A	—	—	—	+	Alk	—	—	A	A	A	A	A	A	—	—	—
	5	A	A	—	—	—	+	Alk	—	—	A	A	A	A	A	A	—	—	—
	6	A	A	—	—	—	+	Alk	—	—	A	A	A	A	A	A	—	A	—
	7	A	A	—	—	—	+	Alk	—	—	A	A	A	A	A	A	—	A	—
	8	A	A	—	—	—	+	Alk	—	—	A	A	A	A	A	A	—	—	—
	9	—	A	—	—	—	—	Alk	—	—	A	A	A	A	A	A	A	—	—
	10	A	A	—	—	—	+	Alk	—	—	A	A	A	A	A	A	—	—	—
10	1	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	A	—
	2	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	A	—
	3	A	A	—	—	—	+	Alk	—	—	A	A	A	—	A	—	—	A	—
	4	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	A	—
	5	A	A	—	—	—	+	Alk	—	—	A	A	A	—	A	—	—	A	—
	6	A	A	—	—	—	+	Alk	—	—	—	A	A	—	A	—	—	A	—
	7	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	A	—	—	—
	8	A	A	—	—	—	+	Alk	—	—	A	A	A	—	A	—	—	A	—
11	1	A	A	—	—	—	+	Alk	—	—	—	A	A	—	A	—	—	A	—
	2	A	A	—	—	—	+	Alk	—	—	A	A	A	—	A	—	—	A	—
	3	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	A	—
	4	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	A	—
	5	A	A	—	—	—	+	Alk	—	—	—	A	A	—	A	—	—	A	—
	6	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	A	—
	7	A	A	—	—	A	+	Alk	—	—	—	A	A	—	A	—	—	A	—
	8	A	A	—	—	—	+	Alk	—	—	—	A	A	—	A	—	—	A	—
	9	A	A	—	—	—	+	Alk	—	—	—	A	A	—	A	—	—	A	—
	10	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	A	—
14	1	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	A	—
	2	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	A	—
	4	A	A	—	—	—	—	Alk	—	—	A	A	A	—	A	—	—	A	—
	5	A	A	—	—	—	—	Alk	—	—	A	A	A	—	A	—	—	A	—
	6	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	A	—
	10	A	A	—	—	—	—	A	—	—	—	A	A	—	A	—	—	A	—
17	1	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	2	A	A	—	—	—	—	Alk	—	—	—	A	A	A	Del	—	—	—	—
	3	A	A	—	—	—	—	Alk	—	—	—	A	A	A	—	—	—	—	—
	4	A	A	—	—	—	—	Alk	—	—	—	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	—	Alk	—	—	—	A	A	A	Del	—	—	—	—
	6	A	A	—	—	—	+	Alk	—	—	—	A	A	A	A	—	—	—	—
	7	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	8	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	—	—	—	—
	9	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	—	—	—	—
	10	A	A	—	—	—	+	Alk	—	—	—	A	A	A	A	—	—	—	—

		Primary Series							Secondary Series											
No. of Case	No. of Strain	Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
19	1	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	—	—	—	—
	2	A	A	—	—	—	—	A	—	—	A	A	A	A	—	—	—	—	—
	3	A	A	—	—	—	+ ?	A	—	—	A	A	A	A	A	Del	—	—	—
	4	A	A	—	—	—	—	A	—	—	—	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	—	A	—	—	—	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	—	Alk	—	—	A	A	A	A	—	—	—	—	—
	7	A	A	—	—	—	—	Alk	—	—	—	A	A	A	—	—	—	—	—
	8	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	9	A	A	—	—	—	—	Alk	—	—	—	A	A	A	—	—	—	—	—
	10	A	A	—	—	—	—	Alk	—	—	—	A	A	A	—	—	—	—	—
22	1	A	A	—	—	—	—	Alk	—	—	A	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	—	Alk	—	—	A	A	A	A	—	—	—	—	—
	3	A	A	—	—	—	—	Alk	—	—	—	A	A	A	—	—	—	—	—
23	1	A	A	—	—	A	—	A & C	—	—	—	A	A	A	A	—	—	—	—
	2	A	A	—	—	—	+	A & C	—	—	—	A	A	A	A	—	—	—	—
	3	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	—	—	—	—
	4	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	5	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	6	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	Del	—	—	—
	7	A	A	—	—	—	—	A & C	—	—	A	A	A	A	A	—	—	—	—
	8	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	9	A	A	—	—	—	+	Alk	—	—	—	A	A	A	A	—	—	—	—
	10	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
24	1	A	A	—	—	—	+	Alk	—	—	A	A	A	A	A	Del	—	—	—
	2	A	A	—	—	—	+	Alk	—	—	A	A	A	A	A	A	—	—	—
	3	A	A	—	—	—	—	A & C	—	—	A	A	A	A	A	—	—	—	A
	4	A	A	—	—	—	+	A & C	—	—	A	A	A	A	A	—	—	—	—
	5	A	A	—	—	A	+	A & C	—	—	—	A	A	A	A	—	—	—	—
	6	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	A	—	—	—
	7	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	—	—	—	—
	8	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	A	—	—	—
	9	A	A	—	—	—	—	A & C	—	—	A	A	A	A	A	A	—	—	—
	10	A	A	—	—	—	+	A	—	—	A	A	A	A	A	—	—	—	—
26	1	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
	3	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
	4	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
	7	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
	8	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
	9	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
	10	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
28	1	A	A	—	—	—	+	A	—	—	—	A	A	A	—	—	—	—	—
	4	A	A	—	—	—	+	A	—	—	A	A	A	A	—	—	—	A	—
	6	A	A	—	—	—	+	A	—	—	A	A	A	—	—	—	—	A	—

		Primary Series							Secondary Series											
No. of Case	No. of Strain	Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
38	1	A	A	—	—	—	+	A	—	—	—	A	A	A	—	—	—	A	—
	2	A	A	—	—	—	+	A	—	—	—	A	A	A	A	—	—	A	—
	3	A	A	—	—	—	+	A	—	—	A	A	A	A	A	—	—	—	—
	4	A	A	—	—	—	+	A	—	—	—	A	A	A	A	—	—	—	—
	5	A	A	—	—	—	+	A	—	—	A	A	A	A	A	—	—	—	—
	6	A	A	—	—	—	+	A	—	—	A	A	A	A	—	—	—	A	—
	7	A	A	—	—	—	+	A	—	—	—	A	A	A	A	—	—	—	—
	8	A	A	—	—	—	+	A	—	—	—	A	A	A	A	—	—	A	—
	9	A	A	—	—	—	+	A	—	—	—	A	A	A	A	—	—	—	—
	10	A	A	—	—	—	+	A	—	—	—	A	A	A	—	—	—	A	—
39	1	A	A	—	—	—	+	A	—	—	A	A	A	—	—	—	—	A	—
	2	A	A	—	—	—	+	A	—	—	—	A	A	A	—	—	—	A	—
	3	A	A	—	—	—	+	A	—	—	—	A	A	—	—	—	—	A	—
	4	A	A	—	—	—	+	A	—	—	—	A	A	—	—	—	—	A	—
	5	A	A	—	—	—	+	A	—	—	—	A	A	—	—	—	—	A	—
	6	A	A	—	—	—	+	A	—	—	—	A	A	—	—	Del	—	A	—
	7	A	A	—	—	—	+	A	—	—	—	A	A	—	—	—	—	A	—
	8	A	A	—	—	—	+	A	—	—	A	A	A	—	—	—	—	A	—
	9	A	A	—	—	—	+	A	—	—	—	A	A	—	—	—	—	A	—
	10	A	A	—	—	—	+	A	—	—	—	A	A	—	—	—	—	A	—
40	1	A	A	—	—	—	+	A	A	—	A	A	A	—	—	—	—	A	—
	2	A	A	—	—	—	+	A	A	—	A	A	A	—	—	—	—	A	—
	3	A	A	—	—	—	+	A	A	—	A	A	A	—	—	—	—	A	—
	4	A	A	—	—	—	+	A	A	—	A	A	A	—	—	—	—	A	—
	5	A	A	—	—	—	+	A	Del	—	A	A	A	—	—	—	—	A	—
	9	A	A	—	—	—	+	A	A	—	A	A	A	—	—	—	—	A	—
	10	A	A	—	—	—	+	A	Del	—	A	A	A	—	—	—	—	A	—
47	1	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	5	A	A	—	—	—	—	Alk	—	—	—	A	A	A	Del	—	—	—	—
	6	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	—	—	—	—
	7	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	A	—
	8	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	9	A	A	—	Del	—	—	Alk	—	—	A	A	A	A	A	—	—	—	—
	10	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
48	1	A	A	—	—	—	+	Alk	A	—	A	A	A	—	—	Del	—	—	—
	2	A	A	—	—	—	+	Alk	A	A	A	A	A	A	—	Del	—	—	—
49	1	A	A	—	—	—	—	Alk	—	—	—	A	A	—	—	—	—	—	A
	2	A	A	—	—	—	—	Alk	—	—	—	A	A	A	Del	—	—	—	—
	3	A	A	—	—	—	—	Alk	—	—	—	A	A	A	—	—	—	—	—
	4	A	A	—	—	—	—	Alk	—	—	—	A	A	—	—	—	—	—	—
	5	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	6	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	7	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	8	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	—	—	—	—

		Primary Series							Secondary Series											
No. of Case	No. of Strain	Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
50	1	A	A	—	—	—	—	Alk	—	—	—	A	A	—	A	—	—	—	—
	2	A	A	—	—	—	—	Alk	—	—	—	A	A	A	A	—	—	—	—
	3	A	A	—	—	—	—	A	—	—	—	A	A	—	A	—	—	—	—
	4	A	A	—	—	—	—	A	—	—	—	A	A	—	A	—	—	—	—
	5	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	6	A	A	—	—	—	—	A	—	—	—	A	A	—	A	—	—	—	—
	7	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	8	A	A	—	—	—	—	A	—	—	—	A	A	—	A	—	—	A	—
	9	A	A	—	—	—	—	A	—	—	—	A	A	—	A	—	—	A	—
	10	A	A	—	—	—	—	A	—	—	—	A	A	—	A	—	—	—	—
51	1	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	2	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	3	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	4	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	5	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	6	A	A	—	—	—	+	A	—	—	—	A	A	A	A	—	—	—	—
	7	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	8	A	A	—	—	—	—	A	—	—	—	A	A	A	A	—	—	—	—
	9	A	A	—	—	—	—	A & C	—	—	—	A	A	A	A	—	—	—	—
55	1	A	A	—	—	—	+	Alk	A	A	A	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	3	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	4	A	A	—	—	—	+	Alk	A	A	A	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	+	Alk	A	A	A	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	7	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	8	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	—	—
	9	A	A	—	—	—	—	Alk	—	—	A	A	A	A	A	—	—	—	—
56	1	A	A	—	—	—	—	A & A C	—	—	A	A	A	A	A	—	—	—	—
64	1	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	3	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	—	—
	4	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	7	A	A	—	—	—	+	Alk	A	—	—	A	A	—	—	—	—	—	—
	8	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	9	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	10	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
65	1	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	+	Alk	A	A	A	A	A	A	—	A	—	—	A
	6	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	Del	—	—	—
	7	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	8	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
67	1	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	3	A	A	—	—	—	+	Alk	A	—	A	A	A	—	—	—	—	—	—
	4	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	8	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	9	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	10	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	—	—

		Primary Series								Secondary Series										
No. of Case	No. of Strain	Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
68	4	A	A	—	—	—	+	Alk	A	—	A	A	A	A	A	—	—	—	—
	5	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
69	1	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	4	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
70	1	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
74	1	A	A	—	—	—	+	Alk	—	A	—	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	3	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	A	—	—	—
	4	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	8	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	9	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	10	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
75	1	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	3	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	4	A	A	—	—	—	+	Alk	—	A	—	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	7	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	9	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	10	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
76	1	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	A	A	—	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	+	A & A C	A	—	A	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	A	A	A	A	A	A	—	—	—	A	—
	7	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	9	A	A	—	—	—	+	A	A	—	A	A	A	A	—	—	—	—	—
78	1	A	A	—	—	—	+	Alk	A	A	A	A	A	—	—	—	—	—	—
	4	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	A	—	—	A	A	A	A	—	—	—	—
	7	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
79	1	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	3	A	A	—	—	—	+	Alk	A	—	—	A	A	A	—	—	—	—	—
	4	A	A	—	—	—	+	Alk	—	—	A	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	A	—
	7	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	—	—
	8	A	A	—	—	—	+	Alk A C	A	—	A	A	A	A	—	—	—	—	—
	9	A	A	—	—	—	+	Alk	A	—	A	A	A	A	—	—	—	—	—

		Primary Series							Secondary Series										
No. of Case	No. of Strain	Glucose	Mannite	Dulcite	Saccharose	Salicin	Indol	Milk—14 days	Sorbite	Rhamnose	Maltose	Levulose	Galactose	Mannose	Raffinose	Dextrin	Inulin	Adonite	Glycerin
82	2	A	A	—	—	—	—	A	—	A	A	A	A	A	A	—	—	A	—
	3	A	A	—	—	—	—	A	—	A	A	A	A	A	A	—	—	A	—
	4	A	A	—	—	—	—	A	—	A	A	A	A	A	A	—	—	A	—
	6	A	A	—	—	—	—	A	—	A	A	A	A	A	A	—	—	A	—
	7	A	A	—	—	—	—	A	—	A	A	A	A	A	A	—	—	A	—
	8	A	A	—	—	—	—	A	—	A	A	A	A	A	A	—	—	A	—
	9	A	A	—	—	—	—	A	—	A	A	A	A	A	A	—	—	A	—
	10	A	A	—	—	—	—	A	—	A	A	A	A	A	A	—	—	A	—
86	1	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	2	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	3	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	4	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	5	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	6	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
	7	A	A	—	—	—	+	Alk	—	—	—	A	A	A	—	—	—	—	—
91	1	A	A	—	—	—	+	A	—	—	—	A	A	—	—	—	—	—	—
	2	A	A	—	—	—	—	A	—	—	A	A	A	—	—	—	—	—	—
	3	A	A	—	—	—	—	A	—	—	A	A	A	—	—	—	—	—	—
	4	A	A	—	—	—	+	A	—	—	—	A	A	—	—	—	—	—	—
	5	A	A	—	—	—	—	A	—	—	—	A	A	—	—	—	—	—	—
	6	A	A	—	—	—	—	A	—	—	—	A	A	—	—	—	—	—	—
	7	A	A	—	—	—	—	A	—	—	A	A	A	—	—	—	—	—	—
	8	A	A	—	A	—	—	A	—	—	—	A	A	—	—	A	—	—	—
	9	A	A	—	—	—	—	A	—	—	—	A	A	—	—	—	—	—	—
	10	A	A	—	—	—	—	A	—	—	A	A	A	—	—	A	—	—	—

APPENDIX II

In Appendix II is given, in a series of tables, the details of all the agglutination tests that were made with 99 strains of the Flexner-Y group all derived from different cases. In these tests only two immune sera were employed, one of which was prepared through the medium of Case 10, Strain 1, and the other of Case 86, Strain 1.

The exact titre of these sera against emulsions of the homologous bacillus appears to have varied at different periods. The following table gives some of the figures found, indicating the titres at various dates.

Serum prepared from Case 10, Strain 1

Date	Titre	
9.6.14	3200 +	
10.7.14	6400 +	
18.8.15	1600 +	3200 P
*14.6.17	4000 +	
30.6.17	1000 +	2000—
25.7.17	1000 +	2000—

Serum prepared from Case 86, Strain 1

Date	Titre	
9.6.14	1600 +	3200 P
9.7.14	6400 +	
18.8.15	3200 +	6400 P
22.9.15	800 +	1600 P
2.10.15	1600 +	3200 P
9.10.15	800 +	1600 P
*14.6.17	2000 +	4000 P
30.6.17	1000 +	2000—

* This was most probably a freshly prepared serum.

In this table and throughout the appendix

\+ indicates complete agglutination.

P indicates partial agglutination.

— indicates that no agglutination took place.

T. S. indicates type strain.

The dates given of the experiments are approximate only; thus if a large series of agglutination tests were made, several days being occupied in carrying them out, the whole series of the tests are classed under the date of the first day.

To these tables is added a column in which the strain of bacillus with which the strain, under test, was found to be in agreement by the absorption test is also given.

TABLE 1

BACILLUS USED IN PREPARATION OF THE SERUM. CASE 10, STRAIN 1

Date of Experiment	9.6.14		10.7.14					2.10.15		20.10.15		9.10.15					20.6.17		Strain with which agreement is shown by absorption test
Dilution of Serum	1 in 400	1 in 800	1 in 400	1 in 800	1 in 1600	1 in 3200	1 in 6400	1 in 400	1 in 800	1 in 400	1 in 800	1 in 50	1 in 100	1 in 200	1 in 400	1 in 800	1 in 500	1 in 1000	Case and
No. of Case and Strain tested																			
Case 10 Strain 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	T. S.
1 5	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
6 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
7 1	+	+	...	...	...	...	...	+	+	+	+	...	...	...	...	...	...	...	10 1
8 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
9 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
11 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
17 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
19 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
22 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
23 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
24 1	+	+	+	+	+	+	+	—	—	+	+	...	...	...	...	...	...	...	10 1
26 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
28 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
38 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
39 1	...	...	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
40 1	+	+	+	+	P	—	—	—	—	—	—	+	+	+	+	+	—	—	170 1
47 1	—	—	...	...	...	...	...	—	—	+	+	+	+	+	+	+	...	...	— —
48 1	...	...	...	...	...	...	...	—	—	—	—	+	+	+	P	—	...	...	86 1
49 1	+	+	+	+	+	+	+	...	...	...	...	...	...	...	...	...	...	...	10 1
50 1	+	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	10 1
51 1	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	...	...	...	10 1
55 1	...	...	...	...	...	...	...	—	—	—	—	+	+	P	—	—	..	...	86 1
56 1	+	—	...	...	...	...	...	+	+	+	+	+	+	+	+	+	...	...	10 1
64 1	...	...	...	...	...	...	...	—	—	+	+	+	+	+	—	—	...	...	86 1
65 1	...	...	...	...	.	...	...	—	—	+	+	...	...	...	...	...	...	...	86 1
67 1	...	...	...	...	...	...	...	—	—	...	...	...	...	...	...	...	..	...	10 1
68 1	..	...	...	...	...	...	...	—	—	...	...	...	...	...	...	...	...	...	86 1
69 1	...	...	...	...	...	...	...	—	—	...	...	...	...	..	...	...	...	...	86 1

When the sign — is placed in this column it indicates that the strain tested did not agree with any of the strains from which the test sera had been prepared; the term 'test strain' indicates that a serum was prepared by means of the bacillus in question.

TABLE 1

BACILLUS USED IN PREPARATION OF THE SERUM. CASE 86, STRAIN 1

Date of Experiment		10.6.14		10.7.14					28.10.14		6.9.15		9.10.15					20.6.17	
Dilution of Serum		1 in 400	1 in 800	1 in 400	1 in 800	1 in 1600	1 in 3200	1 in 6400	1 in 400	1 in 800	1 in 400	1 in 800	1 in 50	1 in 100	1 in 200	1 in 400	1 in 800	1 in 500	1 in 1000
No. of Case and Strain tested																			
Case 10	Strain 1	—	—	...	...	...	...	...	+	+	—	—							
1	5	—	—	...	...	...	...	...	+	+	—	—							
6	1	—	—	...	...	...	...	...	+	—	—	—							
7	1	—	—	...	...	...	...	...	+	+	—	—							
8	1	—	—	...	...	...	...	...	+	+	—	—							
9	1	—	—	...	...	...	...	...	+	+	P	P							
11	1	—	—	...	...	...	...	...	+	+	P	P							
17	1	—	—	...	...	...	...	...	+	+	—	—							
19	1	—	—	...	...	...	...	...	+	+	P	P							
22	1	—	—	...	...	...	...	...	+	+	—	—							
23	1	—	—	...	...	...	...	...	+	+	—	—							
24	1	—	—	...	...	...	...	...	+	+	—	—							
26	1	—	—	...	...	...	...	...	+	+	—	—							
28	1	—	—	...	...	...	...	...	+	+	—	—							
38	1	—	—	...	...	...	...	...	+	+	P	P							
39	1	—	—	...	...	...	...	...	+	+	—	—							
40	1	—	—	...	...	...	...	...	—	—	—	—	...	...	...	...	...	—	—
47	1	—	—	...	...	...	...	...	+	+	P	P	+	+	P	—	—		
48	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
49	1	—	—	...	...	...	...	...	+	+	P	P							
50	1	—	—	...	...	...	...	...	+	+	—	—							
51	1	—	—	...	...	...	...	...	+	+	P	P							
55	1	+	+	+	+	+	+	+	+	+	+	+							
56	1	—	—	...	...	...	...	...	+	+	—	—	+	P	—	—	—		
64	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
65	1	+	+	+	+	+	+	+	+	+	+	+							
67	1	+	+	+	+	+	+	+	+	+									
68	1	+	+	+	+	+	+	+	+	+	+	+							
69	1	+	+	+	+	+	+	+	+	+	+	+							

TABLE 2

BACILLUS USED IN PREPARING THE SERUM. CASE 10, STRAIN 1

Date of Experiment		16.6.14		10.7.14					2.10.14		28.10.15		20.6.17		24.6.17		Case and Strain with which agreement is shown by absorption test	
Dilution of Serum		1 in 400	1 in 800	1 in 400	1 in 800	1 in 1600	1 in 3200	1 in 6400	1 in 400	1 in 800	1 in 400	1 in 800	1 in 500	1 in 1000	1 in 500	1 in 1000		
No. of Case and Strain tested																		
Case 70	Strain 1	...	...	...	...	...	...	...	—	—	...	...	—	—	...	...	86	1
74	6	...	...	...	...	...	...	...	—	—	...	...	...	...	...	...	86	1
75	9	...	...	...	...	...	...	...	—	—	...	...	...	...	...	...	86	1
76	1	...	...	...	...	...	...	...	—	—	...	...	...	...	...	...	86	1
78	7	...	...	...	...	...	...	..	—	—	...	...	...	...	...	...	86	1
79	2	...	...	...	...	...	...	...	—	—	...	...	—	—	...	...	86	1
79a	4	...	...	...	...	...	...	...	...	...	...	...	—	—	...	...	170	1
86	1	...	...	...	...	...	...	...	—	—	...	...	...	...	...	...	T. S.	
91	1	+	+	+	+	+	+	+	...	...	+	+	...	...	...	...	10	1
92	10	+	+	+	+	+	+	+	...	...	+	+	...	...	...	...	10	1
100	1	+	+	+	+	+	+	+	...	...	+	+	...	...	...	...	10	1
102	1	+	+	+	+	+	+	+	...	...	...	...	...	...	...	...	not done	
103	1	—	—	...	...	...	...	...	...	...	...	...	...	...	...	...	not done	
104	1	+	+	+	+	+	+	+	...	...	...	...	...	...	...	...	not done	
113	1	...	...	...	...	...	...	...	...	...	+	+	+	+	...	...	10	1
126	1	...	...	...	...	...	...	...	...	...	...	...	—	—	...	...	86	1
128	1	...	...	...	...	...	...	...	...	...	...	...	—	—	...	...	86	1
130	1	...	...	...	...	...	...	...	...	...	...	...	+	+	...	...	10	1
131	1	...	...	...	...	...	...	...	...	...	+	+	+	+	...	...	10	1
136	1	...	...	...	...	...	...	...	...	...	+	+	+	+	+	+	10	1
137	1	...	...	...	...	...	...	...	—	—	...	...	—	—	—	—	86	1
139	1	...	...	...	...	...	...	...	—	—	...	...	+	+	+	+	10	1
141	1	...	...	...	...	...	...	...	—	—	...	...	—	—	—	—	86	1
144	1	...	...	...	...	...	...	...	—	—	...	...	—	—	—	—	86	1
148	1	...	...	...	...	...	...	...	—	—	...	...	...	...	—	—	86	1
149	1	...	..	...	...	...	...	...	...	...	...	...	—	—	—	—	86	1
154	1	...	...	...	...	...	...	...	...	...	+	+	+	+	+	+	10	1
156	1	...	...	...	...	...	...	...	—	—	...	...	—	—	—	—	170	1
157	1	...	...	...	...	...	...	...	—	—	...	...	—	—	—	—	170	1

TABLE 2

BACILLUS USED IN PREPARING THE SERUM. CASE 86, STRAIN 1

Date of Experiment		10.6.14		9.7.14					28.10.14		6.9.15		20.6.17		24.6.17	
Dilution of Serum		1 in 400	1 in 800	1 in 400	1 in 800	1 in 1600	1 in 3200	1 in 6400	1 in 400	1 in 800	1 in 400	1 in 800	1 in 500	1 in 1000	1 in 500	1 in 1000
No. of Case and Strain tested																
Case 70	Strain 1	+	+	+	+	+	+	+	+	+	+	+	+	+		
74	6	+	+	+	+	+	+	+	+	+	+	+				
75	9	+	+	+	+	+	+	+	+	+	+	+				
76	1	+	+	+	+	+	+	+	+	+	+	+				
78	7	+	+	+	+	+	+	+	+	+						
79	2	+	+	+	+	+	+	+	+	+	+	+	+	+		
79a	4	...	...	...	...	...	...	...	...	...	—	—	—	—		
86	1	+	+	+	+	+	+	+	+	+	+	+				
91	1	—	—	...	...	...	...	...	+	+						
92	10	—	—	...	...	...	...	...	+	+	—	—				
100	1	—	—	...	...	...	...	...	+	+	—	—				
102	1	...	...													
103	1	...	...													
104	1	...	...													
113	1	...	...	...	...	...	...	...	...	...	—	—	—	—		
126	1	...	...	...	...	...	...	...	...	...	+	+	+	+		
128	1	...	...	...	...	...	...	...	...	...	+	+	+	+		
130	1	...	...	...	...	...	...	...	...	...	?	?	—	—		
131	1	...	...	...	...	...	...	...	...	...	—	—	—	—		
136	1	...	...	...	...	...	...	...	...	...	P	P	+	+	+	+
137	1	...	...	...	...	...	...	...	...	...	—	—	—	—	+	+
139	1	...	...	...	...	...	...	...	...	...	?	?	—	—	—	—
141	1	...	...	...	...	...	...	...	...	...	+	+	+	+	+	+
144	1	...	...	...	...	...	...	...	...	...	+	+	+	+	+	+
148	1	...	...	...	...	...	...	..	...	...	—	—	...	...	+	+
149	1	...	...	...	...	...	...	...	...	...	...	...	+	+	+	+
154	1	...	...	...	...	...	...	...	...	...	—	—	—	—	+	+
156	1	...	...	...	...	...	...	...	...	...	P	P	—	—	—	—
157	1	...	...	...	...	...	...	...	...	...	P	P	—	—	—	—

TABLE 3

BACILLUS USED IN PREPARING THE SERUM. CASE 10, STRAIN 1

Date of Experiment	2.10.15		25.6.17		11.9.17		Case and Strain with which agreement is shown by absorption test	
Dilution of Serum	1 in 400	1 in 800	1 in 500	1 in 1000	1 in 500	1 in 1000		
No. of Case and Strain tested								
Case 159 Strain 1	P	P	—	—	...	...	170	1
161	P	P	—	—	—	—	170	1
164	P	P	+	+	...	...	170	1
165	P	P	—	—	—	—	170	1
167	+	+	—	—	...	...	170	1
168	+	+	+	+	...	...	10	1
169	+	+	+	+	...	...	10	1
170	—	—	—	—	—	—	T. S.	
172	—	—	—	—	...	...	311	1
176	...	...	—	—	—	—	86	1
178	...	...	—	—	—	—	86	1
184	—	—	—	—	—	—	170	1
185	...	...	—	—	—	—	86	1
189	...	...	—	—	—	—	86	1
196	...	...	—	—	—	—	86	1
198	P	—	—	—	—	—	—	—
200	—	—	—	—	—	—	311	1
219	—	—	—	—	—	—	311	1
221	...	...	—	—	—	—	86	1
222	...	...	—	—	—	—	86	1
225	—	—	—	—	—	—	311	1
231	—	—	—	—	—	—	311	1
252	—	—	—	—	—	—	275	2
260	—	—	—	—	—	—	311	1
265	—	—	—	—	—	—	—	—
268	—	—	—	—	—	—	311	1
269	+	+	+	+	+	+	10	1
270	+	—	—	—	—	—	311	1
271	—	—	—	—	—	—	86	1

TABLE 4

BACILLUS USED IN PREPARING THE SERUM. CASE 10, STRAIN 1

Date of Experiment	16.6.17		11.9.17		Case and Strain with which agreement is shown by absorption test	
Dilution of Serum	1 in 500	1 in 1000	1 in 500	1 in 1000		
No. of Case and Strain tested					C.	S.
Case 275 Strain	—	—	—	—	T. S.	
290	...	...	—	—	311	1
291	...	...	—	—	86	1
307	+	+	+	+	10	1
311	—	—	—	—	T. S.	
318	+	+	+	+	10	1
319	+	+	+	+	10	1
321	+	+	+	+	10	1
322	+	+	+	+	10	1
325	+	—	+	+	10	1
326	±	±	—	—	86	1
336	+	+	+	+	10	1

TABLE 3

BACILLUS USED IN PREPARING THE SERUM. CASE 86, STRAIN 1

Date of Experiment	6.9.15		25.6.17		11.9 17	
Dilution of Serum	1 in 400	1 in 800	1 in 500	1 in 1000	1 in 500	1 in 1000
No. of Case and Strain tested						
Case 159 Strain 1	P	P	—	—		
161	+	—	—	—	—	—
164	—	—	—	—		
165	P	P	—	—	—	—
167	—	—	—	—		
168	+	—	+	+		
169	—	—	—	—		
170	—	—	—	—	—	—
172	—	—	—	—		
176	+	+	+	+	+	+
178	...	...	+	+	+	+
184	P	P	—	—	—	—
185	+	+	+	+	+	+
189	...	...	+	+	+	+
196	+	+	+	+	+	+
198	—	—	—	—	—	—
200	—	—	—	—	—	—
219	—	—	—	—	—	—
221	+	+	+	+	+	+
222	+	+	+	+	+	+
225	—	—	—	—	—	—
231	—	—	—	—	—	—
252	...	...	—	—	—	—
260	P	P	—	—	—	—
265	P	P	—	—	—	—
268	—	—	—	—	—	—
269	P	P	—	—	—	—
270	+	+	—		—	—
271	P	P	+	+	+	+

TABLE 4

BACILLUS USED IN PREPARING THE SERUM. CASE 86, STRAIN 1

Date of Experiment	16.6.17		11.9.17	
Dilution of Serum	1 in 500	1 in 1000	1 in 500	1 in 1000
No. of Case and Strain tested				
Case 275 Strain	—	—	—	—
290	+	+	—	—
291	+	+	+	+
307	—	—	—	—
311	—	—	—	—
318	—	—	—	—
319	—	—		
321	—	—		
322	—	—	—	—
325	—	—	—	—
326	—	—		
336	—	—	—	—