DIVISIONS OF THE SO CALLED FLEXNER GROUP OF DYSENTERY BACILLI.*

By WILBURT C. DAVISON M.D.

(From the Department of Pediatrics and the Department of Pathology of the Johns Hopkins University, and the Harriet Lane Home of the Johns Hopkins Hospital, Baltimore.)

(Received for publication, June 21, 1920.)

INTRODUCTION.

There is considerable confusion in regard to the existence of subdivisions of the mannitol-fermenting group of dysentery bacilli. Numerous attempts have been made to establish subdivisions on the basis of their biological and serological characteristics (1–8). My conclusions are derived from the fermentation and agglutination reactions of 77 cultures isolated from cases of clinical dysentery in children, 12 cultures from cases of dysentery in the American Expeditionary Forces, and stock cultures of the 5 English types of Flexner bacilli.[1]

By the Flexner group I refer to Gram-negative non-motile bacilli, isolated from the stools of cases of clinical dysentery and agglutinated by the patient's serum. They produce a small amount of indole, do not liquefy gelatin, fail to ferment lactose, and produce acid and no gas in dextrose and mannitol. These are the only constant cultural characteristics. It would seem advisable to apply one name, *Bacillus dysenteriæ* Flexner, to this whole group. These mannitol-fermenting dysentery bacilli have been subdivided in two ways, first by fermentation tests with maltose, saccharose, dulcitol, and rhamnose, and secondly by agglutination tests with the sera of immunized rabbits.

* Read before the 7th annual meeting of the American Association of Immunologists, New York, N. Y., April 1, 1920.

[1] For the technique employed and a description of the cases of dysentery in children see Davison, W. C., Bacillary dysentery in children, *Bull. Johns Hopkins Hosp.*, 1920, xxxi, 225.

Divisions Based on Carbohydrate Fermentation.

In this country it has been customary to refer to the Flexner (9), Harris, Hiss-Russell (10), and Strong (11) divisions on the basis of differences in the fermentation of maltose and saccharose. In my series, by utilizing the differences of fermentation of dulcitol and rhamnose, three additional divisions were found in a few cases (Table I).

TABLE I.

Types of Fermentation of Flexner Dysentery Bacilli.

Fermentation type.	Name.	Motility.	Fermentation of test substances.									Per cent of cases.
			Indole.	Gelatin.	Lactose.	Dextrose.	Mannitol.	Dulcitol.	Rhamnose.	Maltose.	Saccharose.	
1	Hiss-Russell.....	0	+	0	0	+	+	0	0	0	0	7
2	Flexner, English Y....	0	+	0	0	+	+	0	0	+6	0	41
3	Strong.....	0	+	0	0	+	+	0	0	0	+13	14
4	Harris, English V, X..	0	+	0	0	+	+	0	0	+	+15	24
5	English Z.....	0	+	0	0	+	+	0	+12	+4	+12	7
6	*B. alkalescens*.....	0	+	0	0	+	+	+5	0	+	0	3
7		0	+	0	0	+	+	0	+	0	+6	3

If late fermentation occurred, the day of its appearance is designated by the number placed after the + sign. Otherwise + designates acid (no gas) production on the 1st day and indole production on the 7th day. 0 denotes no fermentation (incubation continued 21 days).

Fermentation Type 7 was also isolated from a diarrhea case which was not clinically dysentery.

Other workers using still different carbohydrates have reported additional divisions (6, 12–14). In fact, if fermentation is accepted as a criterion for differentiation, still further members will probably be added in the future as new carbohydrates are used for fermentation tests, and even more confusion will result.

It has been demonstrated (7, 15) that dysentery cultures may lose or gain ability to ferment maltose, saccharose, and also dulcitol (4) and that distinctions made on this basis are inconstant and unreliable. In the same stool I have isolated cultures that fermented maltose and others that failed to do so (Case 53, Table II). In another

TABLE II.

Discrepancies between Fermentation and Agglutination Divisions of Flexner Dysentery Bacilli in Children.

Case No. (Culture No.).	Agglutination type of stool culture.					Summary.	
	Type sera.					Fermentation type.	Agglutination type.
	V	W	X	Y	Z		
53*† (L156), 6* (69, 70, 73)	0	+125	0	±200	0	1	W, Y
6* (72, 74)	±200	0	0	0	0	1	V
39*(L61), 40* (L56, L57, L58)	0	0	0	±200	0	2	Y
39* (L51), 40* (L59), 46* (L82), 53*† (L154), 80* (L11), 10* (161, 174, 175A)	0	0	0	0	0	2	0
46* (L83, L84), 52 (L166, L167), 53*† (L155), 54 (L159)	0	+250	+250	±500	0	2	W, X, Y
80* (L9)	±125	±250	0	±300	0	2	V, W, Y
80* (L10, L12), 81 (L30, L32, L33), 8*† (107, 113, 114, 115, 116), 10* (156, 159, 162, 164, 186, 157)	0	±250	0	±250	0	2	W, Y
9 (153, 154)	+1,250	0	0	0	±500	2	V, Z
Y stock.	+125	0	+250	+625	±100	2	V, X, Y, Z
58 (L174, L175)	0	0	0	0	0	3	0
77 (L186, L187)	0	0	±1,250	+200	±1,250	3	X, Y, Z
5 (67)	+500	0	±200	0	±500	3	V, X, Z
V stock.	+1,250	0	0	+250	0	4	V, Y
X "	0	0	+250	0	0	4	X
41* (L65)	0	0	0	0	0	4	0
41* (L66), 45* (L75, L77), 63* (L114)	0	0	+500	0	+250	4	X, Z
41* (L67)	0	0	0	0	±200	4	Z
41* (L68, L69), 13*† (262, 268)	±200	0	+200	0	+250	4	V, X, Z
45* (L76)	0	+100	+250	±200	0	4	W, X, Y
63* (L115, L116), 78 (L188)	0	0	+500	+200	+150	4	X, Y, Z
79*† (L2, L5), 13*† (261, 263, 266, 270, 271, 274)	±1,000	0	0	0	±250	4	V, Z
13*† (260)	+1,250	0	0	±200	±250	4	V, Y, Z
13*† (267), 79*† (L4)	+500	0	0	0	±500	5	V, Z
Z stock.	0	0	+625	0	+625	5	X, Z
8*† (108)	0	0	0	0	0	6	0
69 (L111, L112), 39 (152)	0	0	0	0	0	7	0

Cases marked with an asterisk (*) had more than one serological type and those marked with a dagger (†) had more than one fermentation type of Flexner dysentery bacillus in the stools. Culture numbers preceded by L, *i.e.* L156, were from cases in Baltimore, the others were from Birmingham, Alabama. + and ± indicate complete and partial agglutination; the numbers following these signs refer to the dilutions (end-point) of the serum. Case 69 was a diarrhea which was not clinically dysentery.

dysentery specimen I have found organisms that differed in dulcitol fermentation (Case 8, Table II). In other stools I have isolated dysentery bacilli that differed in rhamnose fermentation (Cases 13 and 79, Table II).

It is, of course, possible that these represent mixed infections, but this is impossible to prove, inasmuch as the fermentation differences in maltose, saccharose, dulcitol, and rhamnose, as will be pointed out later, do not parallel the serological findings.

Divisions Based on Agglutination with Monovalent Rabbit Sera.

Murray (1) in England has discarded the fermentation divisions of the Flexner group and has found that thirty-four cultures isolated from cases of dysentery in different parts of the world fell into five divisions on the basis of their agglutination reactions with monovalent rabbit sera. These he designates as the V, W, X, Y, and Z divisions of the Flexner group.

As Murray emphasizes, these divisions are not definite but probably indicate that one or more antigens predominate in a given strain. With the English cultures and sera, by means of Dreyer's (16, 17) technique with formolized cultures, I have found that although each serum usually agglutinates its own organism to higher titer than other types (Table III), yet the V serum will also agglutinate the Y culture, the X serum will agglutinate Y and Z, the Y serum will agglutinate the V culture, the Z serum will agglutinate the Y culture, while the W serum, which is of low titer, appears to be specific. Murray states that absorption tests do not make these serological divisions more distinct. It may perhaps be found that agglutination with the sera of animals other than the rabbit may give more clear-cut results, as has been demonstrated (18) with sheep serum for streptococci.

With the cultures which I have isolated there is even more cross-agglutination (Tables II and IV). Some cultures that are agglutinated to end-titer by V serum react to quarter titer with Z. W, X, and Y sera frequently agglutinate the same cultures. With other cultures further combinations were found.

Among 62 cultures from children, of which many reacted with more than one serum (Table II), there were 21 positive agglutination

reactions with V sera, 28 with W, 21 with X, 38 with Y, and 28 with Z, while 15 cultures failed to agglutinate with any of these sera. It is probable that at least 2 of these 15 inagglutinable cultures (Nos. 174 and 175, Tables II and IV) represented a type differing from

TABLE III.

Cross-Agglutination of the Five English Type Sera.

Serum.	Culture.	Agglutination titer.					
		1:100	1:125	1:250	1:625	1:1,250	1:2,500
V	V	+	+	+	+	+	0
	W	0					
	X	0					
	Y	+	+	0			
	Z	0					
W	V	0					
	W	+	+	+	0		
	X	0					
	Y	0					
	Z	0					
X	V	0					
	W	0					
	X	+	+	+	0		
	Y	+	+	+	0		
	Z	+	+	+	+	0	
Y	V	+	+	+	0		
	W	0					
	X	0					
	Y	+	+	+	+	0	
	Z	0					
Z	V	0					
	W	0					
	X	0					
	Y	+	0				
	Z	+	+	+	+	0	

Murray's five, for the patient's serum (Case 58, Table IV) had no agglutinins for the V, W, X, Y, and Z stock cultures. Further artificial cultivation might have rendered the others agglutinable, but the destruction of the laboratory and cultures by fire prevented it.

TABLE IV.

Correlation of Patient's Serum Reactions and Types of Stool Organisms.

Case No.	Length of time after onset.	Patient's serum reactions. Type cultures. V	W	X	Y	Z	No. of culture from stool.	Agglutination type of stool culture. Type sera. V	W	X	Y	Z	Summary. Type of patient's serum reaction.	Agglutination type of stool culture.	Fermentation type of stool culture.
	days														
39	200	0	0	0	±50	0	61	0	0	0	±200	0	Y	Y	2
							51	0	0	0	0	0		0	2
40	205	±200	±200	0	±200	0	56, 57, 58	0	0	0	±200	0	V, W, Y	Y	2
							59	0	0	0	0	0		0	2
46	21	+250	+1,000	+100	+250	±20	83, 84	0	+100	+250	+500	0	V, W, X, Y, Z	W, X, Y	2
							82	0	0	0	0	0		0	2
54	14	+100	0	0	+100	0	159	0	+250	±200	±500	0	V, Y	W, X, Y	2
80	19	+20	±20	0	+200	±20	9	±125	±250	0	±300	0	V, W, Y, Z	V, W, Y	2
	38	+100	±50	0	±200	±20	10, 12	0	±250	0	±250	0		W, Y	2
							11	0	0	0	0	0		0	2
58	6	0	0	0	0	0	174, 175	0	0	0	0	0	0	0	3
77	26	±50	0	0	±50	0	186	0	0	±1,250	+200	±1,250	V, Y	X, Y, Z	3
	76	+20	0	0	0	0	187						V		

41	184	0	±20	0	0	0	66	0	0	+500	0	+250	W	X, Z	4
							68, 69	±200	0	+250	0	+500		V, X, Z	4
							67	0	0	0	0	±150		Z	4
							65	0	0	0	0	0		0	4
63	68	0	0	+20	0	0	115, 116	0	0	+500	+200	+150	X	X, Y, Z	4
							114	0	0	+500	0	±150		X, Z	4
78	13	±20	±20	0	+20	0	188	0	0	+1,250	+500	+500	V, W, Y	X, Y, Z	4
79	17	+200	0	0	+100	+20	2, 5	±1,000	0	0	0	±250	V, Y, Z	V, Z	4
	21	+100	0	0	+100	+50	4	±1,000	0	0	0	±250		V, Z	5
69		0	0	0	0	0	111, 112	0	0	0	0	0	0	0	7

+ and ± indicate complete and partial agglutination; the numbers following these signs refer to the dilutions (end-point) of the serum. 0 indicates no agglutination with the patient's sera at 1:20, and with the diagnostic type sera at 1:150.

In other words, although a distinct division was not obtained, agglutination reactions with the five English type sera and these mannitol-fermenting dysentery bacilli lead to the assumption that different antigens exist and that there are more than five which must be recognized.

Discrepancies between Fermentative and Serological Divisions.

There is no correlation between the divisions by fermentation tests and the serological results. Organisms of the same fermentation reactions were agglutinated by different sera. As an example (Table II), among the group that ferment maltose and saccharose, usually called the Flexner-Harris division (Fermentation Type 4), some cultures were agglutinated only by the Z serum, some by the X, Y, and Z, some by the V and Z, some by the X and Z, some by the W, X, and Y, and still others by the V, X, and Z sera, etc. Conversely, organisms agglutinated by the same sera may have different fermentation reactions. It was also noted that among organisms from the same stool with the same fermentation reactions, some were agglutinated by one or more of the sera while others were inagglutinable.

With the English stock cultures (Tables I and III) the same lack of correlation exists; for instance, the Y and X cultures differ in saccharose fermentation, yet both are agglutinated by the X serum.

Levine (3) has laid emphasis on the fact that the Z culture is the only one to ferment rhamnose, yet it is agglutinated by the X as well as the Z serum. Two rhamnose fermenters isolated from dysentery cases were agglutinated by both V and Z sera. Three rhamnose fermenters, isolated from cases of diarrhea which were not clinical dysentery, were not agglutinated by Z or any of the other sera. Furthermore, the Z serum agglutinated many strains that did not ferment rhamnose. From one stool (Case 79, Table IV) one organism that fermented rhamnose and two that failed to ferment it were agglutinated by V and Z sera.

Dulcitol-fermenting dysentery bacilli from one case were agglutinated by the Y serum and those from another were inagglutinable. This division has been named *Bacillus alkalescens* by Andrewes (2) who regards it as non-pathogenic.

Correlation of Agglutination Reactions of the Patient's Serum and Fermentation and Serological Types of His Stool Organisms.

A partial correspondence exists between the serological type of the organisms isolated from the patient's stool and the agglutination reactions of his serum.

In all except one case (Table IV), if the patient's serum agglutinated any of the five stock antigens, the organisms isolated from the stool reacted with one or more of the sera of the same types. In many cases, however, the stool organisms were not agglutinated by all the types for which the patient had agglutinins. As an example, one patient's serum (Case 40, Table IV) had agglutinins for Types V, W, and Y, while the dysentery bacillus isolated from his stool reacted with only the Type Y serum.[2]

The reverse of this was also true; namely, that the patient's serum would not have agglutinins for all the types which were represented by his stool organisms; for instance, the organisms isolated from a patient's stool (Case 63, Table IV) were agglutinated by the X, Y, and Z sera, while his serum had agglutinins for only the X antigen. These illustrations indicate an overlapping of the antigenic content of these strains.

In the only instance in which there was no correspondence (Case 41, Table IV), the serum of the patient agglutinated the W culture while the organisms from the stool reacted with the V, X, and Z sera.

In a few cases in which tests were repeated after various intervals, if agglutinins persisted, they were of the same types that had been present in previous tests.

CONCLUSIONS.

From these data it is seen that ill defined divisions of the so called Flexner group exist. The divisions do not appear to be sufficiently distinct to warrant the use of separate names. To avoid confusion all mannitol-fermenting dysentery bacilli should be called *Bacillus*

[2] It might be argued that had more colonies been studied in the stool culture, Types V and W might have been found; but this would not explain the instances in which the patient's serum did not have agglutinins for all the types found in his stool.

dysenteriæ Flexner and the subdivision noted. There are two methods for this division, one by the fermentation of carbohydrates, the other by agglutination with monovalent rabbit sera. These do not coincide and one or the other and not both must be adopted. Inasmuch as Murray studied organisms from widely distributed sources, it would seem preferable to adopt his serological classification and to add to it the types that fail to be agglutinated by his V, W, X, Y, and Z sera, as this method is simpler and more rapid. The results of the agglutination reactions of the patient's serum may be expressed in the same terms as the serological typing of the organism from his stool. Fermentation is less constant and gives rise to more divisions than there are carbohydrates.

TABLE V.

Agglutinin Content of Polyvalent Therapeutic Serum.

Serum.	Culture.	Agglutination titer.							
		1:100	1:125	1:250	1:625	1:1,250	1:2,500	1:6,250	1:12,500
Polyvalent therapeutic serum.	V	+	+	+	+	+	+	0	
	W	+	+	+	+	+	+	+	0
	X	+	+	0					
	Y	+	+	+	+	+	+	+	0
	Z	+	+	+	+	+	0		

The serological reactions of these type sera, as Murray points out, show cross-agglutination to a greater or less extent, but they indicate that there are five antigens, V, W, X, Y, and Z and probably others, one or more of which predominate in a given strain. Polyvalent diagnostic and therapeutic sera are practically worthless unless they include antibodies for the more common of these types.

The diagnostic importance of recognizing that there are five or more antigens in this group is seen from the fact that the sera of some patients react with one, others with another, and that unless several antigens are used, some positive tests may be missed.

The therapeutic importance is emphasized by the fact that probably the best polyvalent therapeutic serum at present available has a very low titer for the X antigen, although that type was found in many of these cases (Table V).

BIBLIOGRAPHY.

1. Murray, E. G. D., An attempt at classification of *Bacillus dysenteriæ* based upon an examination of the agglutinating properties of fifty-three strains, *J. Roy. Army Med. Corps*, 1918, xxxi, 257, 353.
2. Andrewes, F. W., Dysentery bacilli: the differentiation of the true dysentery bacilli from allied species, *Lancet*, 1918, i, 560.
3. Levine, M., Dysentery and allied bacilli, *J. Infect. Dis.*, 1920, xxvii, 31.
4. Dudgeon, L. S., et al., Studies of bacillary dysentery occurring in the British forces in Macedonia, *Med. Research Com., Nat. Health Insurance, Special Rep. Series, No. 40*, 1919.
5. Dobell, C., Gettings, H. S., Jepps, M. W., and Stephens, J. B., A study of 1,300 convalescent cases of dysentery from home hospitals; with special reference to the incidence and treatment of amœbic dysentery carriers, *Med. Research Com., Nat. Health Insurance, Special Rep. Series, No. 15*, 1918.
6. Torrey, J. C., A comparative study of dysentery and dysentery-like organisms. Part I, Bacilli producing a typical reaction in litmus milk, *J. Exp. Med.*, 1905, vii, 365; Part II, Dysentery-like organisms which produce an atypical reaction in litmus milk and which should probably be included within the group of pseudo-dysentery bacilli, 385.
7. Sonne, C., Ueber die Bakteriologie der giftarmen Dysenteriebacillen (Paradysenteriebacillen), *Centr. Bakt., 1te Abt., Orig.*, 1915, lxxv, 408. Hehewerth, F. H., Über Dysenterie-Bacillen und ihre Einteilung in Grüppen, *Centr. Bakt., 1te Abt., Orig.*, 1916, lxviii, 3. Thjøtta, T., On the bacteriology of dysentery in Norway, *J. Bact.*, 1919, iv, 355. Gettings, H. S., and Douglas, S. R., An investigation of the Flexner-Y group of dysentery bacilli, *Med. Research Com., Nat. Health Insurance, Special Rep. Series, No. 30*, 1918.
8. Kruse, W., Über die Ruhr als Volkskrankheit und ihren Erreger, *Deutsch. med. Woch.*, 1900, xxvi, 637; Weitere Untersuchungen über die Ruhr und die Ruhrbazillen, 1901, xxvii, 370, 386; Die Blutserumtherapie bei der Dysenterie, 1903, xxix, 49; Zur Geschichte der Ruhrforschung und über Variabilität der Bakterien, 201; Neue Untersuchungen über die Ruhr, 1907, xxxiii, 292, 338; Der jetzige Stand der Dysenteriefrage, *Deutsch. Aerzte-Ztg.*, 1902, i, 25; Die Gesundheitsverhältnisse der Aerzte, Geistlichen und Oberlehrer im Vergleich mit denen anderer Berufe, *Centr. allg. Gsndhtspflg.*, 1900, xix, 229. Dienes, L., Beobactungen über das serologische Verhalten der giftarmen Dysenteriestämme, *Z. Immunitätsforsch., Orig.*, 1919, xxviii, 456. Douglas, S. R., Colebrook, L., and Morgan, W. P., Report upon a series of cases of dysentery received from the Mediterranean Expeditionary Forces, *Med. Research Com., Nat. Health Insurance, Special Rep. Series, No. 6*, 1917. Glynn, E., Berridge, E. M., Foley, V., Price, M., and Robinson, A. L., A report upon 2,360

enteritis "convalescents" received at Liverpool from various expeditionary forces, *Med. Research Com., Nat. Health Insurance, Special Rep. Series, No. 7*, 1918. Park, W. H., personal communication. Park, W. H., Dunham, E. K., Collins, K. R., and Payne, W. A., A clinical and bacteriological study of a number of outbreaks of disease due to the dysentery bacillus of Shiga, *Bull. Med. Sc.*, 1902, ii, 187. Park, W. H., Collins, K. R., and Goodwin, M. E., The dysentery bacillus group and the varieties which should be included in it, *J. Med. Research*, 1904, xi, 553. Park, W. H., and Carey, H. W., The presence of the Shiga variety of dysentery bacilli in an extensive epidemic of dysentery, *J. Med. Research*, 1903, ix, 180. Park, W. H., On the interpretation of reactions of agglutination among the bacilli of dysentery, *Med. Rec.*, 1903, lxiii, 358. Martini, E., and Lentz, O., Ueber die Differenzirung der Ruhrbacillen mittels der Agglutination, *Z. Hyg. u. Infectionskrankh.*, 1902, xli, 540. A study of the serological races of the Flexner group of dysentery bacilli, *Med. Research Com., Nat. Health Insurance, Special Rep. Series, No. 42*, 1919. Winslow, C.-E. A., Kligler, I. J., and Rothberg, W., Studies on the classification of the colon-typhoid group of bacteria with special reference to their fermentative basis, *J. Bact.*, 1919, iv, 429.

9. Flexner, S., On the etiology of tropical dysentery, *Philadelphia Med. J.*, 1900, vi, 414; The etiology of tropical dysentery, *Centr. Bakt., Ite Abt.*, 1900, xxviii, 625; A comparative study of dysenteric bacilli, 1901, xxx, 449; A comparative study of dysentery bacilli, *Brit. Med. J.*, 1901, ii, 786; On the etiology of tropical dysentery, *Bull. Johns Hopkins Hosp.*, 1900, xi, 231. Flexner, S., and Barker, L. F., Report of a special commission sent to the Philippines by the Johns Hopkins University to investigate the prevalent diseases of the islands, *Johns Hopkins Univ. Circ.*, 1900, xix, 13.
10. Hiss, P. H., Jr., and Russell, F. F., A study of a bacillus resembling the bacillus of Shiga, from a case of fatal diarrhœa in a child, *Med. News*, 1903, lxxxii, 289; and *Med. Rec.*, 1903, lxiii, 357; On fermentative and agglutination characters of bacilli of the "dysentery group," *J. Med. Research*, 1904–05, xiii, 1.
11. Strong, R. P., and Musgrave, W. E., Report on the etiology of the dysenteries of Manila, *Rep. Surg.-Gen. Army*, 1900, 251; The bacillus of Philippine dysentery, *J. Am. Med. Assn.*, 1900, xxxv, 498. Strong, R. P., Bacillary dysentery, in Forscheimer, Therapeutics of internal diseases, New York and London, 1914, v, 253.
12. Ruffer, M. A., and Willmore, J. G., On the etiology of dysentery, *Brit. Med. J.*, 1909, ii, 862; The serum treatment of dysentery with notes on the bacteriological diagnosis of dysentery, 1910, ii, 1519.
13. Eyre, J. W. H., Asylum dysentery in relation to *B. dysenteriæ*, *Brit. Med. J.*, 1904, i, 1002.

14. Castellani, A., La malattia del sonno, *Riv. crit. clin. med.*, 1903, iv, 652; On hæmolysins produced by certain bacteria, *Lancet*, 1902, i, 440; Some experiments on the precipitins, 1827; Ueber das Verhältniss der Agglutinine zu den Schutzköpern, *Z. Hyg. u. Infectionskrankh.*, 1901, xxxvii, 381; Some researches on the etiology of dysentery in Ceylon, *J. Hyg.*, 1904, iv, 495. Castellani, A., and Chalmers, A. J., Manual of tropical medicine, London and New York, 2nd edition, 1913, 1380.

15. Aveline, H. T. S., Boycott, A. E., and MacDonald, W. F., *Bacillus dysenteriæ* of Flexner in relation to asylum dysentery, *J. Hyg.*, 1908, viii, 309. Barber, M. A., The variability of certain strains of dysentery bacilli as studied by the single cell method, *Philippine J. Sc., Sect. B*, 1913, viii, 539. Martin, C. J., and Williams, F. E., Types of dysentery bacilli isolated at No. 3 Australian General Hospital, Cairo, March–August, 1916, with observations on the variability of the mannite fermenting groups, *J. Hyg.*, 1917–18, xvi, 257.

16. Dreyer, G., Widal's reaction with sterilised cultures, *J. Path. and Bact.*, 1909, xiii, 331; On Anvendelse af dræbt kultur til Widal-Reaktion, *Hospitalstid.*, 1906, 4. R., xiv, 532; Proceedings of the British Medical Association, Section of Pathology, Discussion on immunity, *Brit. Med. J.*, 1904, ii, 564.

17. Davison, W. C., The diagnosis of enteric fever (typhoid and paratyphoid A and B) by agglutination tests, *J. Am. Med. Assn.*, 1916, lxvi, 1297; Report on prophylactic vaccination with *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B, *J. Lab. and Clin. Med.*, 1916–17, ii, 607.

18. Dochez, A. R., Avery, O. T., and Lancefield, R. C., Studies on the biology of streptococcus. I. Antigenic relationships between strains of *Streptococcus hæmolyticus*, *J. Exp. Med.*, 1919, xxx, 179.