AN ENQUIRY INTO THE *COLI ANAEROGENES* BACTERIA.

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Introduction.

In the routine laboratory examination of specimens of faeces and urine, from time to time certain Gram-negative bacilli belonging to many different groups are encountered, which morphologically show no appreciable differences from the Gram-negative bacilli of the coli-typhoid group, but which however ferment the usual carbohydrate media employed in differentiation with the formation of acid only. Some ferment lactose quickly, others more slowly, and many not at all, but as with the other media no gas is formed. These organisms might be considered as belonging to the so-called *B. coli anaerogenes* group—a somewhat loose term which might include many different strains, some perhaps related to the colon group, others belonging to and related to the group of dysentery bacilli, and others showing no relationship to either.

Lembke (1896) in the faeces of a dog found a colon-like bacillus which was non-motile, produced indol, acidified and clotted milk, but produced only acid in glucose and lactose bouillon. To this organism he gave the name *B. coli anaerogenes*. Since that time a number of other workers have described similar organisms. Dudgeon (1906) found a pathogenic bacillus of this type from the secretion of an enlarged prostate. It produced acid but without gas in lactose, glucose, mannite, maltose and saccharose, acid without clot in milk, and gave a positive indol test. Houston (1903–4) found similar bacilli in the stools of four healthy persons. Cathcart (1906) described similar bacilli among the flora of blown tins of preserved food. Mair (1906), and later Wilson (1908), isolated organisms of this class from cases of cystitis and pyelitis. Castellani (1907) in four cases of continued fever found similar organisms which he considered causative. In two of these cases the organism was obtained by blood culture. In addition Nabarro (1923) isolated a group of organisms which he named *B. coli anaerogenes* from cases of epidemic and other forms of diarrhoea occurring mainly in children. Some of these strains were shown later to be identical with *B. dysenteriae* Sonne.

In this connection also certain type organisms come into consideration, namely, *B. dysenteriae* Sonne, *B. alkalescens* Andrewes and *B. dispar* Andrewes. *B. dysenteriae* Sonne was shown by Sonne (1915) to be the commonest cause of dysentery in Scandinavia, and during the past few years frequently found in dysenteric cases in this country. The cultural and biochemical reactions of

this organism have been intensively studied and may be considered to be well established. It produces acid without gas in 24 hours in glucose, mannite and maltose. Lactose and saccharose are acidified later. Dulcite is not changed and milk is acidified with late coagulation. It does not produce indol. It is thought by some authors that this organism is closely related to *B. dispar* Andrewes, and indeed in the *System of Bacteriology*, Medical Research Council, *B. dispar* Andrewes is given by Gardner (1929) as a synonym of *B. dysenteriae* Sonne.

B. alkalescens described by Andrewes (1918) is a Gram-negative bacillus resembling *B. dysenteriae* Flexner. According to Andrewes it produced acid without gas in glucose, maltose, mannite and dulcite. Lactose and saccharose were not fermented. There were a few strains in which the dulcite reaction was absent and some in which the saccharose was fermented later. Indol was always formed. Alkali was produced with much vigour especially in litmus milk. Smith and Fraser (1928) described a case of continued fever due to this organism.

B. dispar was the name tentatively suggested by Andrewes in 1918 for the lactose-fermenting members of the dysentery group without prejudice as to the number of types or species which may thus be included. According to Andrewes some strains produced acid in lactose early, some later. Acid was produced in glucose, maltose and mannite, sometimes in saccharose and occasionally in dulcite. Acid and clot were formed in milk and some strains produced indol. Twenty-one strains of *B. dispar* Andrewes were investigated by Forsyth (1933), who found that lactose was fermented after 48 hours and that saccharose fermentation preceded it. Dulcite fermentation was irregular and of no value. Indol was produced in peptone water. Milk was acidified and later coagulated. Forsyth found that the dispar group was serologically heterogeneous and as a group distinct from Sonne's bacillus.

It would appear that all of the above-mentioned organisms resembling colon bacilli morphologically but producing acid without gas in carbohydrate media could reasonably be included in a *B. coli anaerogenes* group—those which produce acid in lactose later corresponding to the slow lactose-fermenting type of *B. coli*.

The object of this investigation was to discover the relationship, if any, of these various types of organisms to one another, and also to obtain some indication as to their position in regard to the coli and dysentery groups.

Material for investigation.

For this purpose several groups of organisms have been collected, the grouping being determined by similarity in cultural and biochemical reactions.

Group A. Seven strains of *B. dysenteriae* Sonne have been employed. These strains include two originally isolated by me in 1924 from a small outbreak of dysentery and subsequently shown by Wiseman (1927) to be serologically

identical with *B. dysenteriae* Sonne. Five other strains which have since been obtained in cases of acute dysenteric infection have been included. After 24 hours' growth on plates of litmus lactose agar this organism formed large blue colonies of average diameter 5 mm. The surfaces of the colonies were smooth and the edges for the most part clear cut but beginning to get wavy in places. In 48 hours the colonies were larger and resembled those of *B. alkalescens* Andrewes except that the edges were now wavy and irregular. The blue colour of the centres becomes intensified with further growth. All these seven strains gave the typical biochemical reactions of *B. dysenteriae* Sonne, as mentioned above, with the exception that one strain failed to produce acid in either lactose or saccharose even after two months' incubation. All strains were non-haemolytic and non-motile. In every case the methyl-red reaction tested after incubation for 5 days at 30° C. was positive, though in three cases it was only weakly positive.

Group B. Eight strains of *B. alkalescens* Andrewes have been used, including the type strain from the Lister Institute. All gave the typical biochemical reactions described by Andrewes. At the end of 24 hours their blue colonies on litmus lactose agar can scarcely be distinguished from those of *B. dysenteriae* Sonne, but subsequent growth shows that the centres of the colonies tend to become white and the edges in the majority of cases are clear cut, so that the difference in colonial appearance becomes more evident. The reactions of these eight strains are shown in Table I. It is important to note that all these bacilli were haemolytic, a point of distinction from *B. dysenteriae* Sonne. All were non-motile.

Table I. *Biochemical reactions. Group B*—B. alkalescens *Andrewes.*

Organism	Lactose	Glucose	Mannite	Maltose	Saccharose	Dulcite	Milk	Indol	Haemolysis	Gelatin	Methyl red	Voges-Proskauer	Motility	Source
B. alkalescens Andrewes	alk	a	a	a	alk	a	alk	+	+	−	+	−	−	Type strain from Lister Institute
Williams	alk	a	a	a	alk	a/alk	alk	+	+	−	+	−	−	Faeces—colitis
Hall	alk	a	a	a	alk	a/alk	alk	+	+	−	+	−	−	Faeces—typhoid fever
1114	alk	a	a	a/alk	alk	a/alk	alk	+	+	−	+	−	−	Urine—acute cystitis
Wiggs	alk	a	a	a	alk	a/alk	alk	+	+	−	+	−	−	Faeces—colitis
Millie	alk	a	a	a	alk	a	alk	+	+	−	+	−	−	Urine—cystitis
Jenkins	alk	a	a	a	alk	a	alk	+	+	−	+	−	−	Urine—pyelitis
Pontana	alk	a	a	a	alk	a	alk	+	+	−	+	−	−	Faeces—colitis

a = acidity. alk = alkalinity. a/alk = acidity followed by alkalinity. + = positive. − = negative.

Group C. This group comprises three organisms—the type strain *B. dispar* Andrewes (Lister Institute) and two other strains culturally closely resembling it, "Mew" and "Pearce." All three strains gave blue colonies on plates of litmus lactose agar and showed delayed acid formation in lactose broth. In the case of the type strain and "Pearce" lactose showed acid formation on the third day of incubation, but in the case of "Mew" none appeared until the

tenth day. All three strains gave a positive indol reaction but were non-haemolytic, and non-motile. The reactions of these three strains are given in Table II.

Table II. *Biochemical reactions. Group C*—B. dispar *Andrewes.*

Organism	Lactose	Glucose	Mannite	Maltose	Saccharose	Dulcite	Milk	Indol	Haemolysis	Gelatin	Methyl red	Voges-Proskauer	Motility	Source
B. dispar Andrewes	−a	a	a	a	a	alk	a/ac	+	−	−	+	−	−	Type strain from Liste Institute
"Mew"	−a	a	a	a	alk	a	a/ac	+	−	−	+	−	−	Pus from rectal abscess
"Pearce"	−a	a	a	a	a	alk	a/ac	+	−	−	+	−	−	Faeces—diarrhoea with feve

−a = late acidity. a/ac = acidity followed by coagulation.

Group D comprises four strains all of which form blue colonies on litmus lactose agar and show no change in lactose broth. Their biochemical reactions show a resemblance to those of the Flexner dysentery group, but as will be seen later they are antigenically quite unrelated. All were found to be non-motile. The reactions are shown in Table III.

Table III. *Biochemical reactions. Group D—a group resembling* B. dysenteriae *Flexner.*

Organism	Lactose	Glucose	Mannite	Maltose	Saccharose	Dulcite	Milk	Indol	Haemolysis	Gelatin	Methyl red	Voges-Proskauer	Motility	Source
Kiddell	alk	a	a	alk	a	alk	a/alk	+	−	−	+	−	−	Urine—pyelitis
Bennett	alk	a	a	alk	a	alk	a/alk	+	−	−	+	−	−	Pyosalpinx
Theobald	alk	a	a	alk	a	alk	alk	−	−	−	−	−	−	Urine—cystitis
Apon	alk	a	a	alk	alk	alk	alk	+	−	−	+	−	−	Urine—cystitis

Group E comprises twenty-eight strains which were almost all obtained from cultures of urine or faeces, and of these twenty-four show typical red colon-like colonies on plates of litmus lactose agar and would not be suspected as being other than colonies of colon bacilli. Four strains, however, show quite a different colonial appearance. The strain "Wills" (the last one shown in Table IV) will serve as an example. At the end of 24 hours' incubation on litmus lactose agar a few small pin-point colonies can be seen which after 48 hours are slightly larger and definitely pink in the centre with a clear refractile edge. On plates of simple nutrient agar slightly larger colonies are formed with a granular appearance but in neither case do they approach the size of the colonies in the majority of the organisms in this group or of those of colon bacilli in general. The four strains which form these tiny colonies are indicated in Table IV. With regard to biochemical reactions it will be seen that all show certain features in common. Acid is formed by all in lactose, glucose, maltose and mannite in 24 hours. The reactions in saccharose and dulcite are varied. Almost all give a positive indol reaction and produce acid and clot in milk. Haemolysis is shown

by about one quarter only of these strains. Eight of these twenty-eight strains were found to be motile.

For reasons which will be given later this group will be referred to as *B. coli anaerogenes*.

The reactions are shown in Table IV.

Table IV. *Biochemical reactions. Group E*—B. coli anaerogenes.

Organism	Lactose	Glucose	Mannite	Maltose	Saccharose	Dulcite	Milk	Indol	Haemolysis	Gelatin	Methyl red	Voges-Proskauer	Motility	Source
Plumb	a	a	a	a	a	a/alk	a/ac	+	−	−	+	−	−	Faeces—colitis
Buckley	a	a	a	a	alk	a/alk	ac	+	−	−	+	−	+	Faeces—ulcerative colitis
Frazer	a	a	a	a	alk	a/alk	ac	+	+	−	+	−	−	Urine—cystitis
Joy	a	a	a	a	alk	a/alk	ac	+	−	−	+	−	−	Urine—ureteric calculus
*Simmonds	a	a	a	a	alk	alk	a	−	−	−	+	−	−	Urine—pyelitis
Kata	a	a	a	a	alk	a/alk	a/ac	+	+	−	+	−	−	Urine—chronic pyelitis
9574	a	a	a	a	alk	a/alk	ac	+	−	−	+	−	−	Urine—cystitis
Edwards	a	a	a	a	a	a	a	+	+	−	−	−	−	Urine—chronic appendicitis
Jones	a	a	a	a	a	alk	ac	+	−	−	+	−	−	Faeces—colitis
7562	a	a	a	a	alk	a/alk	ac	+	−	−	+	−	+	Urine—cystitis
T. 2	a	a	a	a	alk	a	ac	+	−	−	+	−	+	Urine—cystitis
5430	a	a	a	a	alk	alk	ac	+	−	−	+	−	+	Gallbladder—cholecystitis
575	a	a	a	a	alk	alk	ac	+	−	−	+	−	−	Urine—pyelitis
578	a	a	a	a	a	a	ac	+	+	−	+	−	+	Urine—pyelitis
760	a	a	a	a	a	alk	ac	−	−	−	−	−	−	Faeces—neurasthenia
Cooke	a	a	a	a	alk	alk	ac	+	−	−	+	−	+	Urine—pyelitis
7568	a	a	a	a	a	a/alk	ac	+	−	−	+	−	−	Urine—cystitis
Frohock	a	a	a	a	alk	alk	ac	+	−	−	+	−	+	Faeces—paratyphoid B fever
Lavell	a	a	a	a	a	a/alk	ac	+	+	−	+	−	−	Urine—cystitis
*Madgewick	a	a	a	a	a	alk	ac	+	−	−	−	−	−	Urine—pyelitis
363	a	a	a	a	alk	alk	ac	+	−	−	+	−	−	Urine—cystitis
Godsome	a	a	a	a	a	alk	ac	+	−	−	+	−	−	Pus—acute arthritis knee-joint
*Bell	a	a	a	a	a	alk	a	+	−	−	+	−	−	Urine—pyonephrosis
8725	a	a	a	a	alk	a/alk	ac	−	−	−	+	−	+	Faeces—neurasthenia
Brett	a	a	a	a	alk	alk	a/ac	+	+	−	+	−	−	Urine—bacilluria
8424	a	a	a	a	a	alk	a/ac	−	+	−	+	−	−	Urine—bacilluria
8829	a	a	a	a	alk	alk	a/ac	+	−	−	+	−	−	Urine—cystitis
*Wills	a	a	a	a	alk	alk	a	−	−	−	−	−	−	Urine—pyelitis of pregnancy

* Very small colonies.

AGGLUTINATION REACTIONS.

Agglutinating sera were prepared against representatives of all the five groups outlined above—against *B. dysenteriae* Sonne (strain "Richards"), *B. alkalescens* Andrewes (Lister Institute strain), *B. dispar* Andrewes (Lister Institute strain), against the organism "Kiddell" selected from Group D, the group resembling *B. dysenteriae* Flexner, and against the organism "Plumb" selected from Group E, *B. coli anaerogenes*. A number of agglutinating sera were also prepared against a number of other strains especially from Group E, and reference to this will be made later. The sera were prepared by the intravenous inoculation of rabbits with increasing doses of saline suspensions of the organisms from live agar cultures, usually beginning with a dose of 100 millions with subsequent doses of 200 and 500 millions at intervals of about five days.

A few days after the third inoculation the serum was tested and was usually found to be of sufficiently high titre for use. Occasionally a fourth injection was required. In no case were any ill effects noted in the animals during the course of these inoculations. In addition the organisms from Groups A, B, C and D were tested against agglutinating sera prepared against the V, W, X, Y and Z strains of Flexner dysentery bacilli. Lastly, in all cases agglutinating tests were carried out with sera prepared against certain strains of *B. coli*. For these sera I am indebted to Professor Dudgeon. They have been prepared against *B. coli* "Dow", a motile and haemolytic strain isolated from the urinary tract, "X 6," a motile and haemolytic strain isolated from the faeces, and "5659," a non-motile organism isolated from a case of acute infection of the urinary tract. This organism "5659" is also haemolytic and belongs to the "slow lactose-fermenting" group (atypical *B. coli*). Of cases of acute urinary infections caused by this group of organisms over 80 per cent. were shown by Dudgeon and Pulvertaft (1927) to be due to organisms of the "5659" type. The agglutinating sera "Dow" and "X 6" were shown by Dudgeon, Wordley and Bawtree (1921, 1922) to agglutinate many colon strains, both haemolytic and non-haemolytic, isolated from urine and faeces, and the serum "5659" agglutinated a considerable number of slow lactose-fermenting *B. coli*, and also many strains of typical *B. coli*. These strains, "Dow," "X 6" and "5659," are considered to be representative but it is realised that the agglutinating power of their antisera is not all embracing because of the innumerable heterogeneous strains of the *B. coli* group.

The antigens employed were saline suspensions of the organisms from live agar cultures. In addition in a large number of cases formalised suspensions of cultures in Dreyer's veal broth were used but as no appreciable advantage was obtained no mention of the results is made in this paper. In the case of Group E, in which group eight strains were motile, alcoholic suspensions were made from all strains both motile and non-motile (except in the case of the strain "Bell"), the latter for control purposes. The growths on agar slopes were removed with absolute alcohol and were kept as thick suspensions in 50 per cent. alcohol. They were diluted with saline solution to a suitable degree when required for the agglutination test. It was found that on applying this method to a culture of the strain "Bell" the suspension which was obtained invariably auto-agglutinated, a difficulty which was not overcome. In the case of all the other strains no such difficulty was experienced. Typical "floccular" and "granular" agglutinations were produced by "Dow" serum against live agar and alcohol-ised suspensions of the "Dow" strain respectively, a fact which testified to the efficacy of the method for eliminating flagellar agglutination.

In carrying out the tests the tubes were incubated in the water bath at 52° C. for at least 5 hours, but it was found in many cases that better results were obtained by incubating overnight, and this was especially necessary in the case of *B. alkalescens* Andrewes and related organisms.

The results of the agglutination reactions are shown in Tables V and VI.

Table V. *Agglutination reactions.*

	Serum Sonne	Serum *alkalescens*	Serum *dispar*	Serum Kiddell	Serum Plumb	Serum Flexner V	Serum Flexner W	Serum Flexner X	Serum Flexner Y	Serum Flexner Z	Serum *coli* "Dow"	Serum *coli* "X6"	Serum *coli* "5659"
Group A. *B. dysenteriae* Sonne:													
Richards	8000	0	100	0	0	0	0	0	0	0	0	0	100
Oliver	8000	0	100	–	0	0	0	0	0	0	0	0	50
Mrs W.	4000	0	100	–	0	0	0	0	0	0	0	0	50
Mr W.	4000	0	100	–	0	0	0	0	0	0	0	0	50
Miller	8000	0	100	–	0	0	0	0	0	0	0	0	100
T. 2	4000	0	100	–	0	0	0	0	0	0	0	0	50
J. L. B.	5000	0	100	–	0	0	0	0	0	0	0	0	50
Group B. *B. alkalescens* Andrewes:													
B. alkalescens	0	1000	0	0	10000	0	0	0	0	0	0	0	0
Williams	0	5000	0	–	5000	0	0	0	0	0	0	0	0
Hall	0	4000	0	–	10000	0	0	0	0	0	100	0	0
8114	0	1000	0	–	10000	0	0	0	0	0	100	0	0
Wiggs	0	4000	0	–	5000	0	0	0	0	0	100	0	0
Lillie	0	2000	0	–	5000	0	0	0	0	0	0	0	0
Jenkins	0	0	0	–	0	0	0	0	0	0	0	0	0
Fontana	0	0	0	–	0	0	0	0	0	0	0	0	0
Group C. *B. dispar* Andrewes:													
B. dispar	0	0	10000	0	0	0	0	0	0	0	0	500	10000
Mew	0	0	1000	0	0	0	0	0	0	100	0	0	250
Pearce	0	0	0	0	0	0	0	0	0	0	0	0	0
Group D:													
Kiddell	0	0	0	1000	0	0	0	0	0	0	0	0	0
Bennett	0	0	0	250	0	0	0	0	0	0	0	0	0
Theobald	0	0	0	0	0	0	0	0	0	0	0	0	0
Apon	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = no agglutination (lowest dilution employed 1 in 25).
– = reaction not tested.

NOTE. With the exception of *B. coli* "Dow" and *B. coli* "X6," which were motile, all the bacilli used for making the antisera were non-motile.

Table VI. *Agglutination reactions*—B. coli anaerogenes.

		Serum Sonne −		Serum *alkalescens* −		Serum *dispar* −		Serum Plumb −		Serum *coli* "Dow" +		Serum *coli* "X6" +		Serum *coli* "5659" −	
	Motility	Alcoh.	L.A.	Alcoh.	L.A.	Alcoh.	L.A.	Alcoh.	L.A.	Alcoh.	L.A.	Alcoh.	L.A.	Alcoh.	L.A.
Group E. *B. coli anaerogenes*:															
Plumb	−	0	0	2000	2000	0	0	5000	10000	0	0	0	0	0	0
Buckley	+	0	0	5000	5000	0	0	10000	10000	50	50	0	0	0	0
Frazer	−	0	0	2000	1000	0	0	1000	2000	0	50	0	0	0	0
Joy	−	0	0	5000	5000	0	0	5000	5000	50	100	0	0	0	0
Simmonds	−	0	0	5000	5000	0	0	5000	5000	50	100	0	0	0	0
Kata	−	0	0	1000	1000	0	0	5000	5000	0	0	0	0	0	0
9574	−	0	0	2000	2000	0	0	10000	5000	50	100	0	0	0	0
Edwards	−	0	0	0	0	0	0	500	500	0	0	0	0	0	0
Jones	−	0	0	100	100	0	0	500	1000	1000	1000	0	0	0	0
7562	+	100	100	100	100	0	0	100	100	0	0	0	0	0	0
T. 2	+	0	0	100	50	0	0	500	1000	250	100	0	100	0	0
5430	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
575	−	0	0	0	0	0	50	0	0	0	0	0	0	0	0
578	+	0	0	0	100	0	0	500	500	10000	10000	0	0	0	0
760	−	0	0	0	0	0	0	0	0	0	0	400	400	0	0
Cooke	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7568	−	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frohock	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lavell	−	0	0	0	0	50	50	100	100	0	0	0	0	0	0
Madgewick	−	0	0	0	0	100	100	0	0	0	0	0	0	0	0
363	−	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Godsome	−	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bell	−	−	400	−	0	−	1000	−	0	−	0	−	0	−	250
8725	+	0	0	0	0	250	400	0	0	0	0	0	0	0	0
Brett	−	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8424	−	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8829	−	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wills	−	0	0	0	0	2000	5000	0	0	0	0	100	100	2000	5000

L.A. = live agar antigen. Alcoh. = alcoholised antigen. − = reaction not tested.

NOTE. Of the organisms used for making the antisera only *B. coli* "Dow" and *B. coli* "X6" were motile.

DISCUSSION OF RESULTS OBTAINED BY CROSS-AGGLUTINATION.

Group A. B. dysenteriae Sonne. The seven strains comprising this group were all agglutinated to a high titre by a single antiserum prepared against one of their number, "Richards". The strain which failed to produce acid in either lactose or saccharose conformed with the others. All seven strains were agglutinated to a very slight extent only by sera prepared against *B. dispar* Andrewes and against a slow lactose-fermenting type of *B. coli*, namely "5659." Sera prepared against the five types of Flexner dysentery bacilli V, W, X, Y, Z, failed to agglutinate any of the seven strains of this group, and conversely serum Sonne did not agglutinate any of the five types of Flexner dysentery bacilli (lowest dilution used 1 in 50).

Group B. B. alkalescens Andrewes. Of the eight strains collected in this group all except two were agglutinated in high dilution by a serum prepared against one of their number, *B. alkalescens* Andrewes (Lister Institute strain). Two strains, "Jenkins" and "Fontana," were not agglutinated by this procedure, but a serum prepared against "Jenkins" agglutinated the homologous organism and *B. alkalescens* each in a dilution of 1 in 2000 and the strain "Fontana" in 1 in 250. It was found that this group is definitely related serologically to a number of organisms contained in Group E (*B. coli anaerogenes*), although the latter are culturally quite dissimilar. This relationship is shown by the results obtained by agglutination with sera prepared against *B. alkalescens* Andrewes, and against the organism "Plumb" chosen from Group E. As will be seen from Tables V and VI, both show slight agglutination with a serum prepared against a haemolytic strain of *B. coli* ("Dow"). Sera prepared against the five different types of Flexner dysentery bacilli did not agglutinate any strain from the alkalescens group, but a serum prepared against *B. alkalescens* Andrewes agglutinated the Flexner strains "V" and "Z" in a dilution of 1 in 50 and "W," "X" and "Y" in a dilution of 1 in 100.

Group C. B. dispar Andrewes. Of the three organisms comprising this group a serum prepared against *B. dispar* Andrewes (Lister Institute strain) agglutinated the homologous organism to a titre of 1 in 10,000 and the strain "Mew" to 1 in 1000. Conversely a serum prepared against the strain "Mew" agglutinated the homologous organism to a titre of 1 in 10,000 and *B. dispar* Andrewes to 1 in 250. The organism "Pearce" was not agglutinated by either of these two sera. It was interesting to find that the organism *B. dispar* Andrewes was agglutinated to full titre $\frac{10000}{10000}$ by the serum prepared against the slow lactose-fermenting *B. coli* "5659" and to a considerably less extent by serum prepared against a typical strain of *B. coli* ("X 6"). It was further shown by absorption tests that the relationship between *B. dispar* Andrewes and *B. coli* "5659" type was a very close one. The results are shown in Table VII.

It was found that the strain "Mew" was also related to the "5659" type of *B. coli*. The strain "Pearce," though culturally showing the same reactions, failed to show any connection.

Table VII. *Reciprocal absorption of serum* B. dispar *and of serum slow lactose-fermenting* B. coli "5659."

	Antigen 5659	Antigen *dispar*
Serum *B. coli* 5659	10000/10000	10000/10000
Serum *B. coli* 5659 absorbed with *B. coli* 5659	0/10000	100/10000
Serum *B. coli* 5659 absorbed with *B. dispar*	500/10000	100/10000
Serum *B. dispar*	10000/10000	10000/10000
Serum *B. dispar* absorbed with *B. dispar*	0/10000	100/10000
Serum *B. dispar* absorbed with *B. coli* 5659	0/10000	2000/10000

Results expressed as a fraction, the denominator showing titre of the unabsorbed serum.

The sera prepared against the dysentery bacilli V, W, X, Y, Z, did not agglutinate any of the three strains of the dispar group except that antiserum Z agglutinated the organism "Mew" in a dilution of 1 in 100.

Group D—a group resembling *B. dysenteriae* Flexner—comprises four organisms, "Kiddell," "Bennett," "Theobald" and "Apon," all with similar biochemical reactions. Antiserum "Kiddell" agglutinated the organism "Bennett" to a slight degree but was without action upon the others. The antisera Flexner V, W, X, Y and Z did not agglutinate any of these four strains, and antiserum "Kiddell" gave slight agglutination with the strains Y and Z only in dilutions of 1 in 50 and 1 in 100 respectively.

Group E—B. coli anaerogenes—comprises twenty-eight strains of which eight were motile. In the case of these eight strains it was found almost invariably that the positive results obtained by using live agar suspensions were closely paralleled by those obtained from alcoholised suspensions. Of the twenty-eight strains comprising this group some were shown to be related to *B. alkalescens* Andrewes and were also agglutinated by a serum prepared against the strain "Plumb" chosen from Group E. The organism "Buckley," which was motile, was agglutinated to the same titre both in live agar and in alcoholised suspensions by both alkalescens and "Plumb" sera, both of which were prepared from non-motile organisms. A number of strains in this group shows very definite affinities with the *B. coli* group. The case of the organism "578" is particularly interesting. As a result of plating out a specimen of urine on litmus lactose agar Professor Dudgeon obtained a growth of red colonies which on inspection appeared to be identical in every respect. Four of these colonies were picked off for further investigation. Two of them, giving the typical reactions of *B. coli* and producing acid and gas in appropriate media, were found by Dudgeon to agglutinate to high titre with serum "Dow." The remaining two were found to produce acid without gas and were regarded as *B. coli anaerogenes*. These were shown by me later to be closely related to *B. coli* "Dow." Serum "Dow" agglutinated live agar and alcoholised suspensions of

the organism "578" to the same titre, 1 in 10,000 producing "floccular" and "granular" clumps respectively, as similarly obtained in the case of the homologous organism. It would appear that the strain "578" is an anaerogenes variant of a typical gas-producing *B. coli* of the "Dow" type. Both are haemolytic. It is also noteworthy that two strains from this group, namely "Bell" and "Wills," also show relationship with *B. dispar* Andrewes and as one would expect with the late lactose-fermenting bacillus "5659." Both these organisms "Bell" and "Wills" are characterised by the formation of very small colonies on litmus lactose agar, quite unlike those of either *B. dispar* or *B. coli* "5659" type, and they form acid in lactose in 24 hours. An agglutinating serum was prepared against the organism "Bell." It agglutinated the homologous organism to a titre of 10,000, the strain "Wills" to 5000, *B. dispar* to 100, the strain "Mew" (Group C) to 1000, but was without effect upon the organism "5659."

Further attempts to differentiate the organisms which have been collected in Group E have been unsuccessful.

Agglutinating sera have been prepared against "Lavell," "Cooke," "8725," and other strains, but no help was obtained as the result of these procedures. The difficulties met with in the serological classification of the *B. coli* group are also encountered in the group *B. coli anaerogenes*, yet it seems reasonable to believe that in Group E there may be found certain types of *B. coli anaerogenes* corresponding to their prototypes in the *B. coli* group proper.

In this connection the work of Penfold (1911, 1911 *a*) and Revis (1911) is of particular interest. It was shown by Penfold that the gas-producing power of *B. coli* could be considerably altered by growth on monochloracetic acid agar. Gas production was completely inhibited in all the sugars fermented by the organism (except isodulcite) but the gas formation from the alcohols (including mannite and dulcite) was retained. By growing the organism in malachite green broth Revis was able to produce an artificial variety from a typical strain of *B. coli*. It was unable to produce gas from any carbohydrate on which it was tested and was unable to clot milk. The condition was quite permanent and growth on gelatin failed to reproduce the original property of gas production.

CONCLUSIONS.

1. The group of organisms known as *B. dysenteriae* Sonne is a well-defined group with typical and distinctive cultural and biochemical reactions. Serologically this group shows only a very slight relationship with *B. dispar* Andrewes and with a haemolytic slow lactose-fermenting type of *B. coli*.

2. The organism *B. dispar* Andrewes is shown by agglutination and absorption tests to be very closely related to a haemolytic slow lactose-fermenting type of *B. coli*, the "5659" type of Dudgeon, and both show definite relationship to certain organisms of the *B. coli anaerogenes* group which are culturally quite dissimilar and produce early acidity in lactose broth.

3. The group of organisms known as *B. alkalescens* Andrewes also shows

typical and distinctive cultural and biochemical reactions. This group is related antigenically with a number of strains of the *B. coli anaerogenes* group and, to a less extent, with the *B. coli* group proper.

4. A small group of organisms (Group D) has been found culturally resembling the Flexner group of dysentery bacilli, but no serological relationship with either the latter or any other group was established.

5. There is a group of bacilli to which the name *B. coli anaerogenes* might be justly applied (Group E). These organisms produce acid in lactose, glucose, mannite and maltose in 24 hours. The reactions in saccharose and dulcite are variable. Gas is not formed in any of these media. Milk is always acidified and frequently coagulated. Most strains form indol in peptone water, and the majority are non-haemolytic. Their biochemical reactions are quite distinct from those of *B. dysenteriae* Sonne and *B. alkalescens* Andrewes. Some strains are serologically related to *B. alkalescens* and some to *B. dispar*, but it can also be shown that a definite relationship exists with the *B. coli* group. It would appear that the group of organisms *B. dispar* Andrewes occupies the same position in relation to this *B. coli anaerogenes* group as the late lactose fermenters do to the *B. coli* group proper.

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