AN INVESTIGATION OF *BACILLUS DYSENTERIAE* (SONNE TYPE III).

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In outbreaks of clinical dysentery, it is often impossible to isolate from the stools of the patients organisms which conform to the classical types of dysentery. Organisms resembling in many ways *B. dysenteriae* Flexner have frequently been met with and form a heterogeneous group, but they nearly all differ from the true Flexner organism in that, early or late, they ferment lactose. The earlier descriptions unfortunately are incomplete and it is now difficult to be certain as to what organism is being described.

Duval and Schorer (1904) from two cases of summer diarrhoea, isolated two varieties of organisms of this type which differed from each other in that only one fermented dextrin. It is not mentioned if the organism produced indol. Torrey (1905) investigated a number of cases of diarrhoea. The stools from these patients were generally of a yellowish green colour and only occasionally contained traces of blood. In no case was a true dysentery bacillus isolated, but organisms of the late lactose-fermenting type were obtained. Indol production was not tested. Kruse (1907) isolated four strains of late lactose-fermenting organisms which he classified as Type E. These organisms were indol positive. Sonne (1914 and 1915) describes a somewhat similar organism as the main cause of dysentery in Copenhagen, and in his classification groups it as Type III. His strains were late lactose fermenters and did not produce indol. D'Herelle (1916) isolated an organism which culturally resembled *B. dysenteriae* Flexner but which was not agglutinated by a polyvalent Flexner serum supplied by the Pasteur Institute. This was probably an inagglutinable strain of Flexner. Lactose was not fermented. Andrewes (1918) tentatively suggested the name *B. dispar* for lactose fermenters of the dysentery group "without prejudice as to the numbers of types or strains which may be thus included." Four of his strains produced indol. Agglutination was vigorous with the acid agglutination test of Michaelis (1915). Andrewes could not get any record of agglutination of these lactose fermenters by the sera of the cases from which they were obtained. Some of the strains were very pathogenic for rabbits. Thjøtta (1919) isolated strains in Norway which agreed with the organism described by Sonne as Type III. None produced indol and all fermented lactose and saccharose. These strains were not agglutinated by normal sera nor by heterologous immune sera but were more sensitive to normal human sera than were typical dysentery organisms. Levine (1920) after investigating a considerable number of strains of late lactose

fermenters of the *dispar* group decided that the indol negative zylose negative variety corresponded with the strain isolated by Sonne in Denmark. Mackie (1919–1920) isolated an indol positive organism of the *dispar* group which was highly pathogenic to rabbits. Thomson and Mackie (1917) described similar organisms, as did also Dean, Adamson, Giles, and Williamson (1917); all their strains were indol positive. Mita (1921) isolated two types of organisms of this group which differed in that one fermented dextrin in 24 hours, while the other required several days to do so. Serologically they were identical. The stools usually contained pus, blood and mucus. Cases that came to autopsy showed a diphtheritic inflammation of the large intestine, which, however, was not so marked as in cases of Shiga and Flexner dysentery. Paterson and Williams (1922) in Australia isolated from a small epidemic of dysentery a late lactose-fermenting organism which they considered to be *B. dysenteriae* (Sonne Type III). Cultures were not tested for indol, nor was zylose used in the fermentation tests. The patients' sera were not tested for agglutinins as the nature of the organism was not recognised in time. The virulence of this organism suggests that it was probably of the Sonne type. Bamforth (1924) investigated a small outbreak of dysentery and isolated an organism culturally identical with Sonne Type III. Smith (1924) described an outbreak of dysentery in Aberdeen and proved by cultural and serological tests that his organism was identical with *B. dysenteriae* (Sonne Type III). Channon (1926) isolated *B. dysenteriae* (Sonne Type III) in England. Her strain was a non-saccharose fermenter. Fyfe (1927) has described a milk-borne epidemic of Sonne dysentery.

The type of illness produced by the Sonne organism is usually mild. Numerous stools are passed daily for a few days and these may or may not contain blood. Mucus and pus are usually present. There is a moderate degree of pyrexia but as a rule the condition of the patient is good and gives little cause for anxiety. Occasionally the disease goes on to a fatal termination. (Paterson and Williams, 1922; Mita, 1921; Glyn and Evans, 1926.)

In the routine examination of stools from children suffering from diarrhoea in the Sick Children's Hospital, Aberdeen, organisms of the Sonne type have been isolated on several occasions. On MacConkey's bile salt neutral red lactose agar plates used for the primary plating of the stools, the colonies were larger than those of *B. dysenteriae* Flexner or Shiga, and frequently showed crenated edges. They could readily be distinguished from the colonies of the paracolon bacillus, which was a common concomitant, in that they were more delicate and translucent and showed the crenated edges. In some cases the organisms were present in large numbers. In all 11 strains were isolated. On inoculation into sugars, glucose and mannite were fermented in 24 hours, and after a number of days, lactose and saccharose also. The organism was non-motile, indol negative, and did not agglutinate with a polyvalent Flexner serum. For comparison, a type strain of *B. dysenteriae* (Sonne Type III (269)) was obtained from the National Collection of Type Cultures, and later, strains

of Mita *paradysenteriae* Types A and B, *B. coli anaerogenes* (1677 and 1931), and *B. dispar*. One strain of *B. coli anaerogenes* isolated in Aberdeen from the urine of a cystitis case (Dickie) was also included in the series. The fermentations are recorded in Table I.

Table I.

Organism tested	Glucose	Lactose	Saccharose	Mannite	Dulcite	Maltose	Dextrin	Zylose	Indol	Motility
10 strains isolated	A	A	A	A	–	A	A	–	–	–
1 strain isolated	A	A	–	A	–	A	A	–	–	–
Sonne 269	A	A	A	A	–	A	A	–	–	–
Mita A	A	A	A	A	–	A	A	–	–	–
Mita B	A	A	A	A	–	A	A	–	–	–
Dispar	A	A	A	A	–	A	A	A	+	–
B. coli anaerogenes (1677)	A	A	A	A	–	A	A	–	–	–
B. coli anaerogenes (1931)	A	A	–	A	A	A	O	O	+	–
B. coli anaerogenes (Dickie)	A	A	–	A	A	A	O	O	+	–

When freshly isolated, all the strains took 10–11 days to ferment lactose and saccharose, but after repeated subculture fermented these sugars in three days. Mita A and B both fermented dextrin in 24 hours. Agglutinating sera were prepared from four of the strains, from Sonne 269, and *B. coli anaerogenes* (1677). It was found that the 11 strains isolated, Sonne 269, Mita A and B, and *B. coli anaerogenes* (1677) were agglutinated to the full titre by each serum, and the absorption of the various sera with these strains removed all agglutinins.

The titres after absorption are recorded in Table II.

Table II.

Serum	Titre	269	Mita A	Mita B	1677	Petrie	Dickson	Matheson	7 strains
Sonne 269	1–2500	nil	nil	nil	nil	nil	nil	nil	nil
Matheson	1–5000	nil	nil	nil	nil	nil	nil	nil	nil
Duncan	1–5000	nil	nil	nil	nil	nil	nil	nil	nil
Dickson	1–10,000	nil	nil	nil	nil	nil	nil	nil	nil
Petrie	1–2500	nil	nil	nil	nil	nil	nil	nil	nil
B. coli anaerogenes (1677)	1–5000	nil	nil	nil	nil	nil	nil	nil	nil

That is, serologically and culturally these organisms are all *B. dysenteriae* (Sonne Type III). The strain of *B. dispar*, *B. coli anaerogenes* (1931), and *B. coli anaerogenes* (Dickie) were not agglutinated by Sonne serum, nor did they remove agglutinins from it. Similarly no strains of Sonne's bacillus agglutinated with *dispar* serum, nor did they absorb the agglutinins from it.

When freshly isolated, none of the 11 strains from the Aberdeen cases were agglutinated by Sonne 269 serum, but they removed all agglutinins from it. After a few subcultures, they agglutinated to full titre with the serum.

With the exception of *B. dysenteriae* Sonne (Dickson), none of these strains were pathogenic for rabbits. The Dickson strain, however, was very virulent and killed rabbits when half an agar slope culture was injected intraperitoneally. The killed culture was as virulent as the living one, and produced

death in less than two hours. The animals showed marked paresis of the hind legs, with quickened breathing within an hour of inoculation. At autopsy the only lesion noted was slight congestion of the small intestine. Virulence was very quickly lost in culture. Feeding the organism to rats produced no effect.

The serum from patients was tested against the isolated organism in three instances and agglutinins were demonstrated in two cases. Serum from Matheson agglutinated up to a dilution of 1–320, and Dickson's serum agglutinated his own strain to 1–160. The third serum had no effect on any strain of Sonne, but it was not obtained till some time after the illness. Normal serum failed to agglutinate any of the strains.

DISCUSSION.

Owing to the fact that they fail to mention indol production, it is impossible to say whether Duval and Schorer (1904) and Torrey (1905) encountered the organism known as *B. dysenteriae* (Sonne Type III). The production of indol recorded by Kruse with his four Type E strains indicates that these were not true Sonne but of the *B. dispar* group. The distinction of two types by Mita on the basis of temporary differences in the rate of dextrin fermentation does not seem to be justifiable. These types are now quite indistinguishable. Serologically they are identical with Sonne Type III. *B. coli anaerogenes* (1677), isolated by Nabarro and obtained from the National Collection of Type Cultures, has been shown culturally and serologically to be a Sonne dysentery bacillus. The 11 strains isolated during this investigation have also been proved to be strains of *B. dysenteriae* (Sonne Type III).

SUMMARY.

B. dysenteriae (Sonne Type III) may be separated from the *dispar* group by the fact that it is indol negative and zylose negative. It is usually inagglutinable when freshly isolated but will remove all agglutinins from a Sonne serum. *B. dysenteriae* (Sonne Type III) is a serological entity and can readily be distinguished from *B. dysenteriae* Flexner and from the *dispar* group. Eleven strains of *B. dysenteriae* (Sonne Type III) have been isolated and examined, along with standard cultures of this group of organisms.

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