

## THE HAEMOLYTIC ORGANISMS OF NORMAL INFANTS' FAECES.

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THE researches of Escherich (1886) show that micro-organisms first appear in the meconium three to seven hours after birth, the subsequent bacterial invasion being progressive up to the third day when it reaches its climax. Escherich isolated 17 varieties of bacilli and cocci, but the principal organisms present at this period were streptococci, *B. coli* and organisms resembling *B. Welchii*. After the third day *B. bifidus* (Tissier) and *B. acidophilus* (Moro) appeared and became the predominant organisms in the faeces of breast-fed infants. Tissier (1900), working with two-hourly samples from the rectum, found that bacteria first appeared in the meconium 10 to 12 hours after birth. Sittler (1909) states that the meconium becomes infected about 12 hours after the birth of the child, the first organism to appear being enterococcus followed by *B. coli* and later by *B. Welchii*. He found that after the third day *B. bifidus* and *B. acidophilus* were the principal organisms in the faeces of breast-fed infants and he sometimes obtained an almost pure culture of *B. bifidus* from infants of this age. Noguchi (1910) states that *B. bifidus* is "an anaerobic phase of life of an aerobic sporogenous organism belonging to the subtiloid group," and he succeeded in transforming the anaerobic *B. bifidus* into an aerobic spored form which he finally again converted to the anaerobic unspored typical form of *B. bifidus*. Passini (1911) found that the age at which bacterial invasion of the intestinal tract occurred was largely dependent on the hygienic surroundings of the infant.

The present investigation was undertaken primarily to determine the frequency with which haemolytic strains of streptococci and *B. coli* occur in normal infants' faeces. The biochemical reactions of all the strains of streptococci which were isolated were also investigated and some observations were made on the occurrence of anaerobic organisms. The infants from whom faeces were obtained were all under one year of age, and at the time when the stools were taken they were in good health, their motions being of normal consistency and appearance. One hundred and one specimens from ninety-one children were examined. The ages of the babies are shown in the following table.

*Haemolytic Organisms in Faeces*

Table I.

Age	Number examined
Under 24 hours	8
On the 2nd day	4
"  3rd  "	6
"  4th  "	11
"  5th  "	15
"  6th  "	8
"  7th  "	3
1 to 2 weeks	21
2 " 3 "	5
3 " 4 "	0
1 " 2 months	4
2 " 3 "	1
3 " 4 "	3
4 " 5 "	2
5 " 6 "	1
6 " 7 "	1
7 " 8 "	3
8 " 9 "	2
9 " 10 "	1
10 " 11 "	2
Total	101

Seventy-eight babies were normal breast-fed infants who, with one exception only, had never been fed by any other method and they received six feeds daily between 5 a.m. and 10 p.m. no feeds being given during the night. Thirteen babies were artificially fed and had previously suffered from malnutrition but they were all in normal health at the time when the stools were examined. The artificially fed babies received mixtures containing varying proportions of raw cow's milk (grade A certified) at the same intervals as the breast-fed infants.

## METHODS.

The stools were at first collected from the unsterilised napkins worn by the babies, but owing to the large number of colonies of *Staphylococcus albus* which were obtained from stools collected by this method, sterile napkins were substituted. As this did not reduce the number of colonies of *S. albus*, the stools were finally collected direct from the rectum into a sterile receiver, but there was no appreciable difference in the results obtained with these various methods of collection.

The stools were dried by Dudgeon's method as detailed by Wordley (1921). A moderately thick layer of faeces was spread on a sterile unglazed porcelain tile and allowed to dry in the air at room temperature under a glass bell jar. Complete drying was assured by transferring the partially dried faeces to a second tile and by this means a fine dry powder was obtained with which suitable culture media were inoculated. The time required for the satisfactory drying of infants' faeces is longer than for the stools of adult persons, but in the majority of cases a fine powder can be scraped from the second tile after three hours' drying. It is very important that the stools should be thoroughly dried, as otherwise the faeces are removed from the tile in the form of flakes instead of as a fine powder, and a confluent growth on the media will probably

result, rendering impossible both the recognition of haemolytic colonies and the sub-cultivation of individual colonies. In four cases in the present series the stools did not dry satisfactorily, probably owing to the presence of an excess of fat, and in these cases the media were inoculated with a sterile swab which had been rubbed over the tile.

Two plates were inoculated with a small quantity of the powder which was thoroughly spread over the surface of the media with a sterile glass spreader. The media used were MacConkey's Bile Salt Lactose Agar and blood agar prepared by adding oxalated human blood to melted agar at 45° C. Some of the powder was placed in a culture tube which contained a small piece of human muscle in glucose broth, and incubated anaerobically at 37° C. for three days in Macintosh and Fildes (1916) anaerobic tin. Another portion of the powder was placed in peptone water and incubated aerobically at 37° C. This culture was used to corroborate results obtained by other methods. The inoculated plates were incubated overnight at 37° C. and examined in the morning, when non-lactose fermenting colonies were picked from MacConkey's medium and their action on the "sugars" investigated. In the case of the blood agar plate, if any haemolytic colonies were present, one of them was sub-cultured on to an agar slope and a non-haemolytic streptococcal colony was also picked whenever these organisms occurred. The action of all the streptococci on human red blood corpuscles, lactose, salicin, inulin, dulcitol, mannitol and litmus milk was tested and the heat resistance of each strain was determined by a method which will be detailed later. The "sugars" were used as a 1 per cent. solution in lemco broth with fuchsin indicator and the tubes were incubated for five days at 37° C. The final test for haemolysis was carried out by the following method as employed by Dudgeon. Each strain of streptococcus and of haemolytic colon bacillus was incubated for 24 hours at 37° C. in 5 c.c. of peptone water to which 0.1 c.c. of solid citrated human red blood corpuscles had been added. Two peptone water cultures prepared in this way were used for each strain, one tube containing 0.5 per cent. and the other 0.85 per cent. of sodium chloride.

The heat resistance of the streptococci was examined by the method employed by Wordley (1921). The organism was cultivated in beef broth for 24 hours and the culture was then placed in a water bath at 60° C. for half an hour at the end of which time it was allowed to cool to room temperature for one hour. An agar slope was then inoculated with three drops of the beef broth culture and after 24 hours' incubation at 37° C. the slope was examined for the presence of streptococcal colonies.

The anaerobic cultures, in glucose broth containing muscle, were incubated for three days in a Mackintosh and Fildes tin at 37° C. Preparations stained by Gram's method were then made and examined microscopically. In 16 cases the anaerobic cultures were further examined by anaerobic sub-culture in litmus milk for 24 hours at 37° C. the presence or absence of the "stormy reaction" characteristic of *B. Welchii* being noted. For this purpose litmus

milk tubes were inoculated with about half a c.c. of the broth culture. The original muscle broth culture was then placed in a water bath at 80° C. for 15 minutes in order to kill all vegetative organisms after which it was again incubated anaerobically for 24 hours. A sub-culture in glucose broth containing muscle was then made and incubated anaerobically for three days, at the end of which time a drop of the culture was plated on agar and incubated anaerobically at 37° C. for three days. Colonies were picked from the agar plate and sub-cultured on to two agar slopes. One of the slopes was incubated anaerobically at 37° C., and the other was incubated aerobically at the same temperature. In those cases in which the organism grew aerobically no further investigation was made, but the obligatory anaerobes were tested for their biochemical reactions.

#### EXAMINATION OF FAECES.

##### I. MICROSCOPICAL.

The faeces of 53 normal infants under one year of age were examined by De Buys and Dwyer (1919) for evidence of parasitic infection with a negative result in every case. In the present investigation, 101 specimens were examined microscopically for the presence of intestinal parasites, a small portion of the fresh stool in strong iodine solution being used, but no entamoebae, cysts, or other parasites were seen. In four cases large starch containing bacilli were seen, one end of the organism being dark blue while the other half had the ordinary refractile appearance. The cultural examination of these four specimens did not result in the isolation of any unusual organisms, they all contained *B. coli*, and Gram positive bacilli were cultivated anaerobically from each specimen, spored forms being seen in two cases.

##### II. BACTERIOLOGICAL.

(a) *Streptococci*. *Streptococcus faecalis* was described by Andrewes and Horder (1906) as a short chain variety which invariably fermented mannitol. They considered *S. faecalis* and *S. salivarius* to be connected by many transitional types, the arbitrary mannitol test being alone used to separate them. Gordon (1921) in his preliminary classification of the streptococci, recognises three main groups as follows:

1. *Pyogenes* or *haemolyticus*, which has no action on neutral red under anaerobic conditions, or on raffinose, seldom ferments mannitol, and produces haemolysis of red blood corpuscles.

2. *Salivarius* or *viridans*, which is non-haemolytic, ferments raffinose, has no action on mannitol, and reduces neutral red in anaerobic cultures.

3. *Faecalis* or *enterococcus*, which is non-haemolytic, has no action on raffinose, ferments mannitol, and reduces neutral red. Houston and McCloy state that *Streptococcus faecalis* is resistant to heat whereas other streptococci are sensitive to heat. Dible (1921) isolated 152 strains of streptococci from

faeces and divided them into two groups. Firstly a diplococcal form, which formed coarse colonies on agar, was able to survive exposure to 60° C. for half an hour, and fermented mannitol; and secondly a variety which formed chains, grew in fine colonies on agar, was not able to resist the heat test, and did not ferment mannitol. Two of the 152 strains isolated by him were haemolytic streptococci and both of them had the characteristics of the chain-forming group. Eighty-five of his strains were heat-resistant and 52 heat-sensitive. Davis (1920) examined the faeces of 53 patients suffering from a variety of diseases few of which, however, were intestinal, but did not find any haemolytic streptococci. Oppenheim (1920) obtained 323 strains of streptococci from the stools of 55 healthy adults, care being taken to avoid any material which was diarrhoeal in character, and he found that five strains had haemolytic properties. Moody and Irons (1920) examined 309 stools from 85 scarlet fever patients and isolated streptococci in 26 cases. Twenty-two strains were found to be haemolytic streptococci which fermented lactose and salicin, but not mannitol or inulin. Wordley (1921), by using Dudgeon's method was able to isolate streptococci from *all* the samples of faeces which he examined. He obtained 52 strains of streptococci from the faeces of 19 adult patients suffering from a variety of diseases and 33 normal persons, and found that 13 of these strains were haemolytic on blood agar and that 11 strains also caused haemolysis of human red cells in peptone water cultures.

In the present series the stools of 101 normal infants both breast and bottle fed were examined and streptococci were found in 65 cases, but only three haemolytic strains occurred. The percentage of cases in which streptococci were found at the various ages are shown in Table II.

Table II.

Percentage of stools from each age period, which contained streptococci, *B. coli*, staphylococci or anaerobic organisms

Age	Number of stools examined in each age period	Anaerobes				
		Streptococci	<i>B. coli</i>	Staphylococci	Unspored	Spored
Under 24 hours	8	12.5	37.5	25.0	0	0
1 to 7 days	47	57.1	89.4	57.4	80	40
1 to 2 weeks	21	57.1	76.2	55.7	62	33
2 to 3 weeks	5	80.0	100.0	60.0	80	20
Over 3 weeks	20	100.0	100.0	45.0	100	10

The three haemolytic strains were obtained from babies aged respectively two hours, two days, and six months. In each of these cases there were only three or four haemolytic streptococcal colonies on the blood agar plate, but many non-haemolytic colonies were also present. The stool of the infant aged two hours which contained haemolytic streptococci was re-examined nine days later but no haemolytic organisms were found at the second examination.

Table III gives the characteristics of all the strains of streptococci which were isolated.

*Haemolytic Organisms in Faeces*

Table III.

No.	Character of colony on agar	Morphology	Lactose	Saiein	Inulin	Dulcitol	Mannitol	Litmus milk	Heat resistance	Haemolysis	Age
2	Coarse	Short chains	+	+	-	+	+	+	-	-	8 months
3	Fine	Chains of 10	+	-	-	-	+	+	+	-	2½ "
4	Coarse	Chains of 8	+	+	-	+	+	+	+	-	9 "
5	Fine	Diplococci	+	+	-	+	+	+	+	-	3½ "
6	"	"	+	+	+	+	+	+	-	-	11 "
7	"	Long chains	+	+	-	+	+	+	+	-	10 "
8	"	Chains of 6	+	+	-	+	+	+	-	-	11 "
11	Coarse	Chains of 10	+	+	-	+	+	+	+	-	9 "
13	Fine	Diplococci	+	+	-	+	+	+	-	+	6 "
14	"	Long chains	+	+	-	+	+	+	-	-	8 "
15	"	Diplococci	-	+	-	-	+	-	+	-	2 "
16	"	"	+	+	-	-	+	+	+	-	4 "
18	"	Chains of 4	+	+	-	-	-	+	-	-	5 "
19	Coarse	Diplococci	+	+	-	-	+	+	+	-	7 "
20	Fine	"	+	+	-	-	+	+	+	-	4 "
21(a)	"	"	+	+	-	-	+	+	+	-	2 hours
21(b)	Coarse	Chains of 6	+	+	-	-	+	+	+	+	2 "
25	Fine	Diplococci	+	+	-	-	+	+	-	-	9 weeks
34	"	Chains of 10	-	+	-	-	-	-	-	+	2 days
37	"	Diplococci	+	+	-	-	+	+	-	-	9 "
38	"	"	+	+	-	-	+	+	+	-	5 "
41	"	"	+	+	-	-	+	+	+	-	5 "
43	"	"	+	+	-	-	+	+	+	-	3 "
46	"	"	+	+	-	-	+	+	+	-	8 "
47	"	"	+	+	-	-	+	+	+	-	9 "
54	"	"	+	+	-	-	+	+	+	-	4 "
55	Coarse	Diplococci	+	+	-	-	+	+	+	-	3 "
61	Fine	Chains of 10	+	+	-	-	-	+	+	-	7 "
64	"	Diplococci	+	+	-	-	+	+	+	-	6 "
68	"	"	+	+	-	-	+	+	+	-	4 "
72	"	"	+	+	-	-	+	+	-	-	9 "
75	"	"	+	+	-	-	+	+	+	-	10 "
76	"	"	+	+	-	-	+	+	+	-	4 "
77	"	Chains of 8	+	+	-	-	+	+	+	-	4 "
81	"	Diplococci	+	+	-	-	+	+	+	-	4 "
82	Coarse	"	+	-	-	-	-	+	-	-	9 "
83	Fine	"	+	+	-	-	+	+	+	-	4 "
84	"	"	+	+	-	+	+	+	-	-	8 months
87	"	"	+	+	-	+	+	+	-	-	2 "
89	Coarse	"	+	-	-	-	-	+	-	-	4 days
91	Fine	"	+	+	-	-	+	+	+	-	3 "
94	"	"	+	+	-	-	+	+	+	-	1 day
95	Coarse	"	+	-	-	-	-	+	-	-	2 days
96	"	"	+	-	-	-	-	+	-	-	6 weeks
98	Fine	"	+	+	-	-	+	+	+	-	4 days
99	"	Chains of 6	+	+	-	-	+	+	+	-	2 "
100	"	Diplococci	+	-	-	-	-	+	+	-	10 "
101	"	"	+	+	-	-	+	+	+	-	10 "
102	"	"	+	+	-	-	+	+	+	-	7 "
103	"	Chains of 6	+	+	-	-	+	+	+	-	18 "
105	"	Diplococci	+	+	-	-	-	+	-	-	11 "
106	"	Chains of 6	+	+	-	-	+	+	+	-	21 "
107	"	Diplococci	+	+	-	-	+	+	+	-	6 "
108	"	Chains of 6	+	+	-	-	-	+	+	-	6 "
109	"	Diplococci	+	+	-	-	+	+	+	-	18 "
110	"	"	+	+	-	-	+	+	+	-	2 "
111	"	Long chains	+	+	-	-	+	+	+	-	3 weeks
114	"	Chains of 6	+	+	-	-	+	+	+	-	5 days

*Abbreviations used in this table.*

- Against "sugar" tubes + means production of acid.  
 " "sugar" tubes - means no change.  
 " litmus milk tubes + means production of acid and clot.  
 " heat resistance + means resistance to exposure to 60° C. for 30 minutes.  
 " heat resistance - means streptococci were killed by exposure to 60° C. for 30 minutes.  
 " haemolysis + means haemolysis on blood agar plate and in peptone water cultures with red cells.  
 " haemolysis - means no haemolysis either on blood agar or in peptone water cultures containing red cells.

It will be seen from this table that 58 strains of streptococci were examined, of which only 48 strains fermented mannitol. Seventy-three per cent. of the strains which fermented mannitol conformed to the type fermentation described by Dible for enterococci, namely, fermentation of lactose, salicin and mannitol, production of acid and clot in litmus milk and no action on inulin or dulcitol. With reference to the heat-resisting properties of those strains which fermented mannitol, it will be seen that 38 strains or 80 per cent. of them were heat-resistant, which is in contrast to the ten strains which did not ferment mannitol for, in their case, only 30 per cent. were heat-resistant. Three haemolytic strains occurred, two of which fermented mannitol and resisted heating at 60° C. for half an hour, the third had no action on mannitol and was killed by heating at 60° C. for half an hour. The diplococcal forms were greatly in the majority; only five strains were isolated, which formed long chains in broth, though there were several cases in which diplococci were accompanied by short chains of four or six cocci.

Table IV gives the percentage of strains which fermented the various "sugars" and for purposes of comparison the corresponding figures are quoted from the various papers which have already been mentioned.

Table IV.

"Sugars"	Percentage of positive fermentations obtained with streptococci from infants' faeces	Percentage of positive fermentations obtained with strains of streptococci isolated from faeces by:		
		Dible	Oppenheim	Wordley
Lactose ...	96.5	78	97	86.5
Salicin ...	89.6	93	97	92.3
Inulin ...	1.7	17	1	9.6
Dulcitol ...	18.9	—	—	7.7
Mannitol ...	82.7	53	76	46.1
Litmus milk (acid and clot)	98.3	72	—	86.5

The streptococci isolated from normal infants' stools are seen, therefore, to correspond with those described as occurring in adults' stools, except in the relatively infrequent occurrence of haemolytic strains, which occurred in only 3 per cent. of cases. This low figure is partly due to the fact that the faeces of some very young babies did not contain any streptococci, either haemolytic or non-haemolytic, but if only those specimens which contained streptococci are considered, haemolytic strains occurred in only 5 per cent. of cases. Wordley (1921) using the same methods, examined the stools of

33 normal adults and found haemolytic streptococci in 15 per cent. of cases; he also examined 19 patients suffering from a variety of diseases and found haemolytic strains in 32 per cent. of cases.

(b) *Bacillus coli*. A paper as yet unpublished by Dudgeon, Wordley and Bawtree (1921) which I am permitted to quote, shows that haemolytic colon bacilli occur in the faeces of normal persons in 13 per cent. of cases. On examining the stools of patients suffering from colitis they found that haemolytic colon bacilli were present in the faeces in 35 per cent. of cases. They also examined the haemolytic properties of strains of *B. coli* obtained from urinary infections and found that the infecting bacilli obtained from men were haemolytic in 76 per cent. of cases, whereas the strains obtained from women were conversely non-haemolytic in 76 per cent. of cases. An anti-serum prepared from one of the haemolytic urinary strains of *B. coli* agglutinated 98 per cent. of the haemolytic strains which they had isolated from urine, and also some of the faecal strains. They also prepared antisera from strains of haemolytic colon bacilli obtained from faeces.

In the present investigation, 101 dried stools were plated on MacConkey's medium and on blood agar and colonies of the colon bacillus resulted in 86 cases, haemolytic colonies being obtained in 12 cases. In the remaining 15 cases there was no growth of *B. coli* on MacConkey's medium, on blood agar or in peptone water. The biochemical reactions of the 12 strains of haemolytic colon bacilli which were isolated were the same in seven cases, namely fermentation with gas production in dextrose, lactose, mannitol, maltose and dulcitol, no change in cane sugar, florescence in neutral red broth, indol formation, and production of acid and clot in litmus milk (*B. coli communis*). Three strains in addition to the above reactions produced acid and gas in cane sugar (*B. coli communior*). The remaining strains did not ferment dulcitol or cane sugar but were in other respects the same as the first group (*B. acidilactici*). The organism known as *B. lactis aerogenes* did not occur. Dudgeon, Wordley and Bawtree in the course of their work previously referred to, examined eight of these strains of haemolytic colon bacilli and found that six of them were agglutinated by their antisera and that the two remaining strains were not agglutinated by the antisera which they had prepared. The ages of the babies from whose stools haemolytic colon bacilli were isolated are given in Table V.

Table V.

Age	Method of feeding	Number of stools which contained haemolytic <i>B. coli</i>
2 days	Breast	1
3 "	"	1
4 "	"	3
5 "	"	1
10 "	"	1
19 "	"	1
4 months	Bottle	2
5 "	"	1
11 "	"	1
		12

It will be seen from this table that eight strains of haemolytic colon bacilli were obtained from the faeces of 78 breast-fed infants and that four strains were obtained from 13 artificially-fed babies. The percentage of occurrence of haemolytic colon bacilli was therefore 10·3 per cent. for breast-fed babies and 30·8 per cent. for bottle-fed infants.

In addition to the twelve strains of haemolytic colon bacilli four strains of haemolytic coliform bacilli were isolated, but as they did not conform to any recognised type they need not be further discussed.

(c) *Staphylococcus albus*. *S. albus* was very frequently observed, being present in 55 stools out of 101 examined. *S. aureus* did not occur in a single instance. Twelve strains of *S. albus* were haemolytic on blood agar, the colonies being surrounded by a large clear area which gave the appearance of intense haemolysis but when these strains were cultivated with red blood corpuscles in peptone water the degree of haemolysis produced was found to be very small and in some cases no haemolysis was observed. In the majority of these cases there was a profuse growth of *S. albus* on the blood agar plate and in four cases this organism was found in pure culture. The haemolytic colonies usually occurred in association with non-haemolytic colonies of the same organism but in four cases the haemolytic variety was found alone. The organisms were examined microscopically in every case to make sure that colonies of Sarcinae were not mistaken for *S. albus*. It would appear from these observations that *S. albus* occurs much more frequently and in greater abundance in infants' faeces than in adult stools.

(d) *Anaerobic organisms*. One hundred and one anaerobic cultures were examined and Gram positive organisms were seen in 65 cases. Twenty-nine of these cultures contained spore forming bacilli. The ages at which they occurred are shown in Table II. In 16 cases the anaerobic cultures obtained from the faeces of babies of varying ages from two days to six weeks were further examined as to their action on litmus milk, but the "stormy reaction" characteristic of *B. Welchii* was not obtained in any of these cases. The 16 cultures were also plated as described previously and single colonies picked from the plates. Only two of the colonies picked were obligatory anaerobes, one being a slender Gram negative bacillus with oval terminal spores, and the other a thick Gram positive bacillus with sub-terminal spores. A large number of spored organisms which grew aerobically and anaerobically equally well were also found. The Gram positive spored organism did not ferment dextrose, lactose, maltose, mannitol, cane sugar, dulcitol or inulin, litmus milk was acidified and clotted, the clot being unchanged, and coagulated serum was not digested. In the case of the Gram negative organism dextrose and lactose were fermented with gas production, other sugars and litmus milk were unchanged and coagulated serum was not digested.



stools which contained *B. coli* gradually increased from day to day, but that the occurrence of streptococci and staphylococci was more variable. The youngest baby from whom a haemolytic strain of streptococcus was isolated was two hours old, a haemolytic strain of *B. coli* was obtained from a baby on the third day after birth, and a haemolytic strain of staphylococcus was isolated on the fourth day.

## SUMMARY.

1. No entamoebae, cysts, or other parasites were found.
2. Streptococci were present in the stools of all babies more than three weeks old, but only three haemolytic strains were isolated, which appears to be a lower proportion than occurs in adult stools.
3. Colon bacilli were present in the stools of all babies more than two weeks old, and haemolytic strains occurred in 12 per cent. of the specimens examined, that is in the same proportion as in adult stools.
4. *Staphylococcus albus* was much more commonly found than in adult stools and in greater abundance.
5. There was no bacteriological difference between the stools of the 78 breast-fed babies and those of the 13 artificially-fed infants, except that the haemolytic strains of *B. coli* occurred relatively more frequently in the case of the artificially-fed infants but owing to the small number of haemolytic strains isolated it would be unwise to lay too much stress on these figures.

In conclusion I wish to offer my sincere thanks to Dr Jewesbury, Physician to the Children's Department of St Thomas's Hospital, for supplying me with the necessary material and for information as to methods of feeding, and I am also greatly indebted to Professor Dudgeon and Dr Wordley for their constant help and advice.

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