BACTERIOLOGICAL INVESTIGATION INTO THE CAUSATION OF DIARRHOEA AND ENTERITIS IN DUBLIN IN 1942–3

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(With 1 Figure in the Text)

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PART I. INVESTIGATION OF MATERIAL OBTAINED IN DUBLIN

INTRODUCTION

The term 'diarrhoea and enteritis under 2 years' is used to describe a universal condition, responsible for a high mortality in infants, of which the common features are diarrhoea and dehydration.

Various bacilli, including *Proteus vulgaris*, *B. morgani*, dysentery bacilli and paracolon bacilli, have been regarded from time to time as the causative organisms, while in some outbreaks no 'abnormal' bacteria have been found, and a virus suspected.

PRESENT INVESTIGATION

In 1941 diarrhoea became prevalent in Dublin, causing 506 infant deaths, double the number ascribed to this cause in 1940. It seemed possible that this diarrhoea was bacterial in origin, particularly as McNally (1942) reported the isolation of the Newcastle bacillus from two affected children.

The present investigation, which commenced in June 1942, under a full-time grant from the Medical Research Council of Ireland, was instituted on the advice of a special committe of medical men set up by the Department of Local Government and Public Health.

'Diarrhoea and enteritis' became notifiable in Dublin in March 1942. Dr J. St L. O'Dea, who was investigating the clinical, epidemiological and sanitary aspects of the disease, visited the cases at various hospitals and at their homes, and collected specimens of faeces, etc., which were brought to the laboratory.

Four hundred cases were investigated, from which 418 specimens were examined, namely, 278 rectal swabs and 140 specimens of faeces, of which 50 were in Sachs's alkaline glycerine saline (Sachs, 1939). One hundred rectal swabs from 100 controls were also examined. On arrival, each rectal swab was spread on to one or more plates, so as to ensure well-isolated colonies. Washed flakes of mucus from the faeces were spread on to plates. These were incubated at 37° C. and examined after 24 and 48 hr., when colonies were picked off for identification. The media used for the routine plating of the specimens were human blood agar, and either MacConkey's medium or Hynes's (1942) modification of Leifson's (1935) desoxycholate medium or both. A small series of cases was investigated for *Cl. Welchii* spores.

A. General analysis of cases and controls

The majority of the patients were severely ill, since 64 % were admitted to hospital and 36 % are known to have died. Clinically, the 400 cases were classified into 'mild' (18 %), 'moderate' (24 %) and 'severe' (58 %), according to the general condition of the patient and the degree of dehydration present.

At the time of collection the specimens were classified into 'early', within 4 days of the commencement of the illness, 'intermediate', between the fifth and tenth days, and 'late', after the tenth day. Neglecting second specimens from some cases, 100 cases were classified as 'early'. 158 as 'intermediate' and 142 as 'late'.

Of the cases examined 62 were neonatal, 278 under 6 months and 36 under 2 years. There were 220 males and 180 females. Of the 100 controls, 4 were under 1 month, 72 under 6 months and 10 infants. There were 52 males and 48 females.

Eighty of the specimens from the cases were plated within 30 min. of collection, 170 within 60 min. and 96 within 90 min. Of the specimens from the controls 26 were plated within 30 min., 68 within 60 min. and 13 within 90 min.

Of the control specimens 95 % were from healthy children attending various Child Welfare clinics, the remainder were from hospital cases. Children free from intestinal upsets and a history of diarrhoea were chosen.* Selection to obtain a similar proportion of artificially fed infants in both series eliminated the bogey of comparing results from diarrhoeal, artificially fed infants with those of breast-fed controls. 92 % of 272 cases under 6 months and 93·4 of 72 controls under 6 months were artificially fed.

The cases and controls were therefore comparable in age-sex distribution, manner of feeding and the ages of the specimens before plating.

B. Types of organisms isolated

The following organisms, *Proteus vulgaris*, paracolon bacilli, *B. morgani*, dysentery bacilli, *Ps. pyocyanea*, staphylococci and *Cl. welchii*, were isolated as shown in Table 1.

Table 1. *Showing the frequency of isolation of suspected bacteria from* 400 *cases of 'diarrhoea and enteritis' and from* 100 *non-contact controls*

	Cases %	Controls %
Proteus	45–52	16
Paracolon bacilli: Actual	26·2	11
Probable	39	
B. morgani	6·5	2
Staphylococcus pyogenes	3·5	3
Haemolytic *Staphylococcus albus*	10	40
Dysentery bacilli	3·75	0
Ps. pyocyanea	0·75	0
None of the above isolated	30·5	—

Cl. welchii: 25 % of 12 cases examined

C. Direct microscopic examination of faeces

Faeces from forty cases were examined microscopically. Neither helminth ova nor protozoa were ever seen. Pus cells were invariably present (10–30 per high-power field). Red blood corpuscles were usually few, one or two per four or five fields, and were often absent.

* In spite of these precautions, two of the controls from whom paracolon bacilli were isolated had positive histories of 'diarrhoea and enteritis' about 2–3 months previously, when the mothers were subsequently re-questioned.

D. DYSENTERY BACILLI

(1) *Technique used for isolation and identification*

After 24 or 48 hr. incubation, non-lactose-fermenting colonies, if present, were picked off the MacConkey or desoxycholate media.

After incubation overnight specimens from the agar cultures were stained by Gram's method and subcultures made into lactose. Next day growth from the lactose culture was subcultured into glucose, maltose, mannite, dulcite and saccharose,* broth, gelatin (stab culture) and on to one or more agar slants. Sometimes tubes of litmus milk were inoculated.

The biochemical investigations were performed in Durham tubes containing 1 % of the test 'sugar' in peptone water (dulcite only 0·5 %) containing Andrade's indicator. These were incubated for 10–14 days and examined daily. Motility was determined by the hanging drop method in an 8–24 hr. broth culture. Indole production in broth was tested for by Ehrlich's aldehyde reagent after 24, 48 and 72 hr. Gelatin stabs were grown at room temperature.

(2) *Serological identification*

Preliminary slide agglutinations were followed by macroscopic tests, the bacterial suspensions for which were made in 0·5 % carbol saline from overnight slope cultures, when non-viable, saline dilutions to opacity 400 million *B. coli* per c.c. were made. The Sonne, Newcastle and various Flexner dysentery antisera were those of the Oxford Standard Laboratories (titre 1/250). The anti-*alkalescens* (Towne) serum was prepared in a rabbit (titre 1/2500).

Final serum dilutions of 1/25, 1/50, 1/125, 1/250 and a control all in normal saline were set up, using the drop method. Often the dilutions ranged to 1/5000, particularly when *B. alkalescens* was suspected. The tubes were placed in a water-bath at 52–55° C. for 16–18 hr., when they were removed, allowed to stand for 2–4 hr. and read with the agglutinoscope.

Results. Fifteen strains of dysentery bacilli were isolated from the 400 cases (3·75 %), namely, Newcastle (two strains), Sonne (five strains), *alkalescens* (two strains) and Flexner (six strains: 1 Type V, 3 Type W, 1 Type X, 1 Type Z).

This incidence of 3·75 % is small when compared with many results reported from the U.S.A. and elsewhere (Duval & Basset, 1904; Wollstein & Dewey, 1904; Bioca & Cammarella, 1939; Denison & de Holl, 1935), but other workers report a comparable incidence (e.g. Fothergill, 1929; de Roda, 1939).

Two Sonne strains were isolated from sisters and a third came from an infant in the same street. This fact suggests that Sonne was responsible for a small local outbreak of three cases, and was probably the cause in two isolated cases. The other dysentery bacilli were isolated from children living mostly in widely separate areas.

No dysentery bacilli were isolated from the 100 controls.

A clinical analysis revealed no distinguishing mildness, severity or fatality in this small group of cases.

* Occasionally xylose and sorbite were also used.

One of two conclusions must be reached: either that in a small percentage of the cases in Dublin dysentry bacilli were responsible for 'diarrhoea and enteritis', or that some children were carriers of these pathogenic organisms, which, however, did not cause the disease. The negative results in the control series seem to support the former view.

E. NON-LACTOSE-FERMENTING BACILLI, OTHER THAN *B. DYSENTERIAE* AND *PROTEUS*

Non-lactose-fermenting colonies resembling the Salmonella-dysentery type were seen on many plates. Often they were very numerous, outnumbering the pink *Bact. coli* colonies, but in many plates only a few pale colonies could be seen. Some of these colonies yielded *Ps. pyocyanea*, *B. morgani* and paracolon bacilli.

(1) *Ps. pyocyanea*

Only three strains of this bacillus were isolated from 400 cases (0·75 %) and none from the 100 controls. Presumably it was of little significance.

(2) *B. morgani* No. 1

Twenty-six strains of *B. morgani* were isolated from 400 cases (6·5 %) and two strains from 100 controls (2 %). Since, however, both MacConkey's medium and Hynes's desoxycholate medium inhibit the growth of this organism (Hynes, 1942), the latter more than the former, a non-inhibitory differential medium, such as litmus lactose-agar, should have been used to establish its real incidence.

In only five of the twenty-six cases from which it was isolated was *B. morgani* the only non-lactose-fermenting bacillus present. In fifteen cases a strain of *Proteus* was also found.

Morgan & Ledingham (1909) described this bacillus as the cause of summer diarrhoea in London. The present investigation sheds no light on its role in Dublin.

(3) *Proteus*

(*a*) *Isolation and identification*

Human blood agar was used for the isolation of *Proteus* organisms as they spread freely on this medium, and the presence of Gram-negative bacilli in a preparation from the spreading edge was regarded as sufficient to identify then. Occasionally, when blood agar was not used, the differential medium for non-lactose-fermenters was employed. Colonies of non-lactose-fermenting Gram-negative bacilli which 'swarmed' over the agar slant subculture were regarded as *Proteus*.

Strains of *Proteus* were isolated from 180 (45 %) out of 400 cases. In 340 cases blood agar was used as a medium and *Proteus* was found on 176 of these. The higher figure (51·8 %) in the latter cultures was probably a truer index of its frequency.

From the 100 control cases, *Proteus* was isolated sixteen times (16 %).

The biochemical and metabolic activity of 40 *Proteus* strains was investigated, thirty-one from cases, nine from controls. The various maltose + and −, indole + and − types were present in both groups.

(*b*) *Analysis of the cases from which* Proteus *organisms were isolated*

To ascertain whether the presence of *Proteus* was associated with the duration or severity of the disease, the results of the 340 cases in which blood agar was used were analysed. In 176 cases *Proteus* was found and in 164 it was not found.

The first analysis showed that the isolation of *Proteus* did not depend on the stage of the disease.

Prof. O'Meara suggests that the association of *Proteus* with infants in the first 6 months of life may be due to physiological changes in the intestine.

Table 3 is an analysis of the cases in age groups, with reference to severity and fatality, and it shows that the isolation of *Proteus* from cases aged 1–3 months (and 0–12 months) is associated with severity and fatality.

The report continues: 'It seemed at first that the relationship between *Proteus* and severity for some age groups might have been a consequence of the strong though irregular association between *Proteus* and age. This, however, does not appear to be the case.'

Table 2. Proteus *and age*

	0–1 month	1–3 months	3–6 months	6–12 months	12 months	Total
Cases:						
Proteus group	34	81	39	20	2	176
Non-*Proteus* group	33	31	24	44	32	164
Total	67	112	63	64	34	340
Controls:						
Proteus group	0	9	4	3	0	16
Non-*Proteus* group	4	26	33	17	4	84
Total	4	35	37	20	4	100

$\chi^2 = 60{\cdot}97, \quad N = 4$

Table 3. Proteus, *severity and fatality*

	Mild	Moderate	Severe	Total	χ^2	Result	Deaths	Survivors	χ^2	Result
0–1 month:										
Proteus	2	7	25	34	0·29	$0{\cdot}9 > P > 0{\cdot}8$	21	13	0·12	$0{\cdot}8 > P > 0{\cdot}7$
Non-*Proteus*	3	6	24	33		Not significant	18	15		Not significant
Total	5	13	49	67			39	28		
1–3 months:										
Proteus	5	9	67	81	12·44	$P < 0{\cdot}01$	50	31	10·19	$P < 0{\cdot}01$
Non-*Proteus*	0	12	19	31		Significant	8	23		Significant
Total	5	21	86	112			58	54		
3–6 months:										
Proteus	4	9	26	39	2·77	$0{\cdot}3 > P > 0{\cdot}2$	19	20	3·79	$0{\cdot}1 > P > 0{\cdot}05$
Non-*Proteus*	6	6	12	24		Not significant	5	19		Probably not significant
Total	10	15	38	63			24	39		
6–12 months:										
Proteus	2	6	12	20	5·99	$P < 0{\cdot}05$	2	18	0·004	$P = 0{\cdot}95$
Non-*Proteus*	13	18	13	44		Significance doubtful	3	41		Not significant
Total	15	24	25	64			5	59		
0–12 months	Total value of χ^2 for $N = 8$				21·49	$P < 0{\cdot}01$ Significant	Total value of χ^2 for $N = 4$		14·1	$P < 0{\cdot}01$ Significant

Table 2 gives the age distribution of the infants for the cases and controls in both *Proteus*-positive and *Proteus*-negative groups. These and other figures were submitted to the Statistics Branch, Department of Industry and Commerce, for analysis. The Report commences:

'Significance is tested throughout by χ^2 (Chi-squared)

'*Result.* Difference between the *Proteus* and non-*Proteus* distributions are overwhelmingly significant.... The large value χ^2 is due to the excess in the *Proteus* group at ages 1–6 months and deficiency at ages over 6 months.'

Metchnikoff (1914) isolated *Proteus vulgaris* from 93 of 218 cases of infantile diarrhoea in Paris. He fed chimpanzees on the faeces of the infants, produced diarrhoea, and found *Proteus* in the faeces of the affected apes.

It seemed important therefore to discover whether the *Proteus* strains isolated could produce diarrhoea in experimental animals.

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(c) *Animal feeding experiments with* Proteus

Seven healthy baby rabbits (4–5 weeks old, weighing from 8 oz. to 1 lb.) were used. Three controls were fed by pipette with 1 c.c. of sterile broth and the four others with 1 c.c. of a 24 hr. broth culture of recently isolated *Proteus* strains (two from the cases and two from the controls). All the rabbits were observed for 3 weeks. The control rabbits remained well, no diarrhoea appeared and they gained weight rapidly. The experimental rabbits remained well, but they increased in weight more slowly than the control group.

Generally speaking, the results were negative, but very few animals and only the rabbit species were used.

(d) *Examination of the sera of patients from whom strains of* Proteus vulgaris *and/or* B. morgani *no.* 1 *were isolated*

The sera from eleven patients were examined. Each serum was tested for agglutinins against various suspensions in saline in final dilutions of 1/25, 1/50, 1/125, 1/250 and a control. The *Proteus* and *B. morgani* no. 1 suspensions were boiled to destroy flagellar agglutinins. Suspensions of paracolon and other organisms were also used, namely, 42(1) (type A), 78(*a*) (type AB Alk C), 237(1) (type Dc), 115(1) (type Cd), 247(*x*) (type Ea), *B. dysenteriae* Newcastle, Sonne and one or more strains of Flexner.

Negative results were obtained with the paracolon, Newcastle, Flexner and Morgan's *no.* 1 *suspensions* and with all except one of the *Proteus* O suspensions (in this the titre was low, i.e. 1/25 trace). Seven sera were tested against *B. dysenteriae* Sonne, and four were positive, two of the titres reaching 1/125 and 1/250 respectively.

Remembering that the patients were 3 weeks to 5 months old, that the duration of illness was from 4 days to 1 month, negative results are not inconsistent with the pathogenicity of *Proteus vulgaris* or *B. morgani*. The titres obtained against *B. dysenteriae* Sonne are peculiar because in no case was this organism isolated. In this connexion the agglutination given by paracolon bacilli against dysentery anti-sera is of interest (see Part II).

(4) *Paracolon bacilli*

The bacteriology of paracolon bacilli is described in Part II, but a short summary may be useful here. The paracolon bacilli are Gram-negative rods resembling *B. coli*, but fail to ferment lactose or ferment it late. Most strains are classifiable according to their fermentation of dulcite and saccharose; glucose, maltose and mannite generally being fermented with acid and gas production. Group I (the dulcite +, saccharose − strains) is biochemically stable, and serologically most strains are identical (Type A). Moreover, this group particularly is antigenically related to one or more of the dysentery bacilli, and a subgroup was found strongly related to *B. alkalescens*. Group II (dulcite −, saccharose −) contains serologically heterogeneous strains, though some strains can be included in the A, B, C, D, E antigenic pattern. Group III (dulcite +, saccharose +) is biochemically a stable group and contains a subgroup of non-maltose-fermenters. Serologically it is divided into three main groups, types Cd, Dc and Ea, leaving a number of antigenically heterogeneous strains. Group IV (dulcite −, saccharose +) hardly deserves a place as a group, as biochemically many of its members are unstable and serologically it is heterogeneous.

Numerous investigators have isolated paracolon bacilli from the faeces of infants with infectious diarrhoea. Morgan & Ledingham (1909), though they attributed the disease to Morgan's no. 1 bacillus, also isolated paracolon bacilli from a large proportion of cases.

Fothergill (1929) reluctantly suggested that the paracolon strains which he isolated from 44 % of cases in Boston caused the disease. The strains isolated by Pisu (1939), corresponding to Group I bacilli, produced a fatal enteritis in rabbits when injected intravenously. Dudgeon (1925) found paracolon bacilli in only 2 % of normal stools, but Sandiford (1935) in Egypt, who found them in 16·1 % of stools sent in for a diagnosis of dysentery and in 8·6 % of normal stools, concluded that their presence was without significance.

The results of the present investigation require critical analysis to assess the significance of the paracolon bacilli isolated. The analysis proceeds as follows: (*a*) Frequency of isolation of paracolon bacilli in the cases and controls. (*b*) Antigenic structure of the paracolon bacilli and their relationship to the dysentery bacilli. (*c*) Examination of patients' sera for agglutinins. (*d*) The reproduction of diarrhoea in the experimental animal. (*e*) Examination of specimens of milk and flies for paracolon bacilli.

(*a*) *Frequency of isolation*

Altogether 114 paracolon strains were isolated from 105 cases (26·25 %). However, in the first 306 cases MacConkey's medium was used, and in the later 175 Hynes's desoxycholate medium. Paracolon bacilli were isolated almost twice as frequently on Hynes's medium.

Another incidence, the 'probable' frequency, may then be calculated:

Paracolon strains in the first 225 cases	42
Multiply 42 by factor 2 to obtain probable incidence	84
Paracolon strains isolated in the later 175 cases	72
Total	156

∴ 'Probable' frequency of isolation is 39 % (156 out of 400 cases).

Hynes's desoxycholate medium was used for all controls and eleven strains of paracolon bacilli were isolated (11 %).

Similar 'actual' and 'probable' frequencies were calculated for paracolon Group I, II, III and IV organisms isolated from the cases. These are given in Table 4 contrasted with the frequency in the control series.

There was no doubt that paracolon bacilli were more frequently isolated from cases of infantile diarrhoea than from the non-contact controls. This was particularly true for Group I bacilli. A further analysis referring to the antigenic structure of the organisms showed that strains containing the A antigen and those included in the A, B, C, D, E antigenic pattern are more likely to be of significance than other strains. Moreover, at least three of the eleven positive controls were under suspicion, as the positive results might have been due to incubation of the disease in one case, or the carrier condition in the other two.

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The records were analysed to see if severity and fatality were related to the isolation of paracolon bacilli (cf. *Proteus*), but no correlation was found.

(*b*) *Antigenic structure of the paracolon bacilli and their relationship to the dysentery bacilli*

Most Group I strains belonged to a single bacterial species, and their most important antigen (A) occurred frequently in Group III strains and occasionally in the other groups. Moreover, these organisms and others included in the A, B, C, D, E classification are related to the dysentery bacilli, particularly the Flexner, Newcastle and *alkalescens* types, whilst in general those outside this classification are not so related. Remembering the diarrhoea of adult bacillary dysentery, it seems reasonable to suspect organisms isolated from a diarrhoeal condition in infants which are related serologically to the dysentery bacilli. The Newcastle bacillus was also found to be related serologically to the Flexner group of bacilli, and is now admitted to the dysentery group.

This argument supports the suggestion that many paracolon strains are capable of causing diarrhoea in infants.

(*c*) *Examination of patients' sera for agglutinins against the paracolon bacilli*

Practical difficulties arose which prevented the examination of more than nineteen sera. The serum dilutions used were 1/25, 1/50, 1/125 and 1/250, and each was tested against the paracolon strain from the case and against other members of the same group.

Sera from sixteen patients, twelve under 6 months of age, from whom Group I Type A strains had been isolated, were tested. Seven of these were negative (titre 1/25). The remainder were positive, against the patients' strains and serologically related strains, the titres varying from 1/25 and 1/250, usually 1/50 or 1/125. Five positive results were from patients who had been ill 4–6 weeks, three negative results from patients who had been ill 2–3 weeks, and two more from patients who had been ill 6–8 weeks. The number of tests was small, but agglutinins seem more likely to be present from the fourth to the sixth week of illness than at any other time. Assuming that the paracolon bacillus isolated was the cause of the diarrhoea, then, on the analogy of enteric fever, agglutinins against this organism should not be detectable in the early stage of the disease, though they should make their appearance later on. On the other hand, in bacillary dysentery agglutinins are often absent at all stages of the disease. Moreover, the antibody-forming apparatus of young infants is immature, and therefore negative results cannot be used as an argument against the pathogenicity of these bacilli, positive results alone being significant.

Only three other sera were examined and with negative results.

Of the 400 cases in the present series only three are known to have had otorrhoea. From the stools and the ear discharge of one of these Group I Type A bacilli were isolated.

(*d*) *Reproduction of the disease in the experimental animal*

Feeding experiments with young rabbits. Rabbits, 4–6 weeks old, weighing 8–24 oz., kept in separate cages, were used. With these, two series of experiments were performed. In the first series nine rabbits were divided into two groups of three control and six experimental animals. The latter were fed by pipette with 1 c.c. of a 24 hr. broth culture of a paracolon strain and the controls with 1 c.c. of sterile broth. Five strains were Group I bacilli, one was a Group II bacillus. The animals were examined daily and weighed every few days. Two rabbits died, one within 48 hr., the other in 12 days. Autopsies revealed a mild enteritis and a bilateral pneumonia in the former, and a mild enteritis with enlarged mesenteric glands in the latter. Neither had diarrhoea. Bacteriological examinations showed the infecting organisms in the spleen, aortic glands, and contents of the small intestine of the former, but were negative in the latter. The other four experimental rabbits remained well.

Table 4

Series	Group I		Group II		Group III		Group IV		Other paracolon bacilli	
	Actual %	Probable %	Actual %	Probable %	Actual %	Probable %	Actual %	Probable %	Actual %	Probable %
Cases (400)	12	16·7	3·2	4·2	7·2	9·5	2	2·75	4·5	6
Controls (100)	1*		1		6		3		0	

* The infant from whom this organism was isolated vomited a few days after the specimen was collected and passed some green motions.

The controls were kept in a different animal house to avoid possible cross-infection. They remained well and put on weight rapidly.

After 14 days two animals used as controls were fed with Group I Type A strains. One was unaffected, but the other died in 48 hr. Autopsy revealed a mild gastro-entero-colitis, but all cultures gave negative results.

A second series of rabbit experiments was carried out on ten animals. Seven were fed with 1 c.c. of a 24 hr. broth culture of a strain of the paracolon bacillus, five with Group I Type A 1, one with Group II Type Ac, and one with Group III Type Ea, and three controls with 1 c.c. of broth. No rabbit developed diarrhoea and all remained healthy.

In the two series three out of fifteen young rabbits fed with large doses of actively growing paracolon bacilli died, but from one only was the organism recovered, and this rabbit died rapidly of pneumonia. No animal suffered from diarrhoea, although the three animals showed post-mortem evidence of enteritis. The results were therefore inconclusive.

Feeding experiments with kittens. The procedure was the same as in the rabbit experiments. The kittens' milk was boiled to minimize outside sources of infection.

In the first series of experiments seven young kittens, weighing from 7 to 20 oz., were used. Two fed with sterile broth served as controls. Four were fed with 1 c.c. of a 24 hr. broth culture of various Group I Type A bacilli and one with a Group II Type Ac bacillus. Three developed severe diarrhoea in 24 hr., and 5 and 8 days and died in 2, 12, and 14 days respectively, the last two thin and dehydrated. Autopsies revealed, in the first, a definite ileo-colitis with injected mesentery and enlarged mesenteric lymph glands; in the second, a severe haemorrhagic ileo-colitis with melaena and enlarged mesenteric glands; and in the third a petechial ileo-colitis with enlarged mesenteric glands. Before death the infecting organisms were found in the stools, and post-mortem the bacilli were isolated from the spleen and mesenteric glands in two cases and from the mucus of the large intestine in all three. The fourth kitten had a transient attack of diarrhoea on the tenth day. The fifth never had diarrhoea, but failed to gain weight. Bacteriologically their faeces were negative on several occasions. The controls remained healthy.

Two weeks later both controls were fed with 1 c.c. of *B.* Clarke broth culture (Group I Type A). On the next day one had diarrhoea which continued, and 8 days later the kitten was dehydrated and appeared moribund. Paresis of all four limbs had developed, particularly the forelimbs (left > right). Meanwhile the organism was isolated from the stools. The other kitten remained well. Both were killed on the 22nd day. In the first kitten autopsy revealed a *haemorrhagic gastritis* with semi-digested blood in the stomach, a mild enteritis and a definite petechial colitis with enlarged mesenteric glands, and in the second no abnormal changes except enlarged glands in the mesentery. Bacteriological investigation revealed the infecting organism in the mesenteric glands of the first kitten.

In the second series, eight kittens were used. Three of them (weighing 20–30 oz.) were fed with 0·1 c.c. of broth culture of Group I Type A organisms (in a volume of 1 c.c.). Two never suffered from diarrhoea, but in 2 weeks their weights had fallen by 3 or 4 oz. The infecting organisms were not isolated from their faeces. The third had diarrhoea in 4 days, but this cleared up a week later. Group I Type A bacilli were found in its faeces.

Three others kittens (each weighing 16 oz.) were fed with 10 million, twice-washed bacilli from 24 hr. agar-slope cultures (Group I Type A, Group II unclassifiable, and Group III Type Dc respectively). The first developed diarrhoea in 3 days and lost weight, and the infecting organism was isolated from its stools. The other two remained healthy.

The two controls, fed with sterile broth, remained well.

In the first series four out of seven kittens developed diarrhoea due to an entero-colitis when fed with large doses of actively growing Group I Type A bacilli. These organisms were isolated from the faeces during life and from the spleen and/or mesenteric lymph glands at post-mortem. In the second series two of six kittens developed diarrhoea. One had been given a smaller dose of bacilli, the other saline-washed organisms, and in both the infecting organism was present in the stool. The kittens resistant to the action of the bacteria were usually older and heavier than those which proved susceptible.

The condition produced in kittens resembled the 'diarrhoea and enteritis' of infants, particularly in severity, loss of weight, and dehydration.

Koch's postulates have therefore been fulfilled with Group I Type A bacilli.

(5) *Examination of specimens of milk and flies for non-lactose-fermenting Gram-negative bacilli*

If 'diarrhoea and enteritis' is an enteral bacterial infection, these bacteria must find their way from the faeces of a patient or carrier to another infant. Possible means of transference are milk and flies.

Thirty specimens of milk were examined for non-lactose fermenters. Fifteen were obtained from the homes of infants suffering from diarrhoea, the others from various dairies in the city. Of the latter five were samples from T.T. herds supplying Infant Aid Depots, six were 'bottled' pasteurized milk, and the remainder were 'loose' milks, non-pasteurized and non-tuberculin-tested.

Results. 'Gelatin-liquefying Group IV' strains were isolated from two specimens of milk, but their significance is doubtful. No known pathogens or strains of Paracolon Groups I, II, or III were found, but one Group IV strain was isolated.

Flies from four houses where cases had occurred were examined for non-lactose fermenters. From one group an 'inagglutinable Flexner' strain, probably of no significance, was isolated.

In general the results of cultures from milk specimens and flies were negative, but only a small number of specimens was examined.

F. ISOLATION OF STAPHYLOCOCCI

Theoretically, 'diarrhoea and enteritis' in infants could be due to enterotoxin-producing strains of staphylococci gaining access to the milk of the artificially fed infant, or occurring in breast milk if the breast is inflamed or the nipple is sore.

Blood agar was used for the isolation of staphylococci, and all plates containing haemolytic *Staph. aureus* or numerous staphylococci were considered possibly significant. In practice only those plates containing an occasional non-haemolytic *Staph. albus* were ignored, as such organisms were probably saprophytes.

The staphylococci were classified into *Staph. albus* and *pyogenes* varieties according to the coagulase test, chromogenicity and haemolysin production.

Frequency of isolation. From the 400 cases, 54 strains of staphylococci were isolated (13·5 %), of which 14 (3·5 %) were *Staph. pyogenes.* The 100 controls yielded 43 strains of staphylococci (43 %), 3 of which were *Staph. pyogenes* (3·%).

It appears surprising that staphylococci were more frequently isolated from normal stools than from diarrhoeal cases. But it is likely that many from the former were anal or rectal strains, as the rectal swab is likely to be contaminated with the flora of the normal dry rectum, but the anus of the diarrhoeal infant,

through which loose faeces are frequently passing, is less likely to be contaminated. The control series may then be disregarded when considering staphylococci. Are the staphylococci from the diarrhoeal stools significant? The only way of assessing a strain is to test its entero-toxin-producing powers. This was not done.

Case analysis showed that the isolation of staphylococci was not related to clinical severity or fatality.

G. The incidence of *Cl. welchii*

Lamb dysentery is due to *Cl. welchii* (Type B), the pathogenity of which depends on an exotoxin (B), distinct from the gas-gangrene-producing toxin. 'Diarrhoea and enteritis' of infants in some ways resembles lamb dysentery. Faeces of cases of infantile diarrhoea were therefore examined for *Cl. welchii*.

Technique. A modification of Brewer's (1940) method of growing anaerobes 'aerobically' was used.

Thioglycollic acid was added to boiled, sterile broth to give a concentration of 0·05 %.

An emulsion of the faeces was heated to 80° C. for 10–15 min. to kill the vegetative forms of bacteria, and two loopfuls inoculated into a tube of thioglycollic broth. This was incubated at 37° C. and inspected for growth after 24, 48, and 72 hr. Absence of growth after 3 days' incubation was considered a negative result. If stout Gram-positive bacilli or suspicious organisms were present, the culture was plated out on blood agar and incubated anaerobically for 48 hr. Subcultures were made from suspicious colonies consisting of stout Gram-positive rods into various media for diagnosis.

Results. Twelve specimens of faeces were examined. Seven showed growth in thioglycollic broth, but only three grew *Cl. welchii*. No strain was tested for entero-toxin production, but in view of the greater frequency of *Cl. welchii* in normal faeces it is unlikely to have had any aetiological relationship to diarrhoea and enteritis.

SUMMARY

(1) The results of a bacteriological investigation of the faeces of 400 cases of 'diarrhoea and enteritis' occurring in young children in Dublin in 1942–3 are described, and are compared with those obtained from 100 comparable non-contact controls.

(2) The possible pathogenic agents isolated were *Proteus vulgaris*, dysentery bacilli, paracolon bacilli, staphylococci, *B. morgani* and *Cl. welchii*.

(3) *Proteus* was isolated from about 50 % of cases as against 16 % of controls. The correlation between the isolation of *Proteus* and the severity and fatality of the disease, particularly in the 1–3 months age group, was also in favour of its pathogenicity.

(4) Dysentery bacilli were isolated from 3·75 % of the cases, but none from the controls. The strains isolated were *Bact.* Sonne, *Bact.* Newcastle, various types of *Bact.* Flexneri and *Bact. alkalescens*. These can probably cause infantile diarrhoea.

(5) *Paracolon bacilli.* The 'probable' incidence was 39 % from the cases as against 11 % from the controls. Of the strains isolated from the cases 94 % were classifiable into Groups I–IV, as were all the strains from the controls.

The following evidence is produced in support of the view that paracolon bacilli may have been the causative organisms:

(*a*) Of Groups I–IV 70 % are classifiable by serological methods. Group I Type A is a single bacterial species.

(*b*) Most strains are antigenically related to the dysentery bacilli.

(*c*) The results of the examination of patients' sera for agglutinins suggest that Group I Type A strains are pathogenic.

(*d*) Feeding experiments with paracolon bacilli produced in young kittens an entero-colitis resembling in many ways the 'diarrhoea and enteritis' of infants. The bacilli were isolated from the faeces of the kittens which contracted diarrhoea and also from the intestinal contents and organs at post-mortem.

(6) Staphylococci were isolated from 13·5 % of the cases. No strain was tested for enterotoxin production.

(7) The methods adopted inhibited the isolation of *B. morgani*, and it was isolated in only 6·5 % of cases and 2 % of controls.

8. *Cl. welchii* was present in 25 % of twelve diarrhoeal stools, but these positive results were probably without significance.

PART II. THE BACTERIOLOGY OF PARACOLON BACILLI

INTRODUCTION

The paracolon bacilli are Gram-negative rods of the genus *Bacterium* which either fail to ferment lactose or ferment it late or irregularly. They attack other 'sugars', however, producing acid or acid and gas. Biochemically they usually differ from the *Salmonella* and dysentery groups. They differ from *Proteus* in failing to spread on solid media, if motile. Most strains fail to liquefy gelatin and do not produce H_2S. Most paracolon bacilli closely resemble the *coli-aerogenes* group, but differ from members of that group in their failure or slowness to attack lactose. Consequently, they are often called non- or late lactose-fermenting *Bact. coli*.

The classification of these bacilli has presented great difficulties. Dugdeon (1924) and later Dudgeon & Pulvertaft (1927) classified 300 strains on the basis of the fermentation of dulcite and saccharose, 86 % fermenting dulcite but not saccharose and most of these were serologically of the same type (Group A). Numerous other workers (Pisu, 1939; Jones, Orcult & Little, 1931; Kennedy, Cummings and Morrow, 1932; Fothergill, 1929), who have studied these organisms, have found difficulty in classifying them.

Clayton & Warren (1929) described a non-lactose-fermenting gas-forming bacillus as causing dysentery and called it the Newcastle bacillus. This organism is now classified as a dysentery bacillus. Probably it was

formerly included amongst the unrecognized, apparently unimportant, paracolon bacilli, and was the first of them to be assigned a pathogenic role and a definite name.

BACTERIOLOGICAL FINDINGS

In the following pages the investigation of 108 strains of paracolon bacilli, mostly isolated from cases of diarrhoea and enteritis, is described.

A. CULTURAL INVESTIGATIONS

All the strains were Gram-negative rods, morphologically indistinguishable from *Bact. coli*. In conditions of growth, type of growth in broth and on gelatin and agar, and resistance to heat and phenol, the strains did not differ from the *coli-aerogenes* group.

(1) *Metabolic and biochemical activity*

All strains fermented glucose and mannite and nearly all maltose with the production of acid and gas. Lactose was never fermented in 24 hr. Some strains failed to ferment it in 10–14 days, but most were late lactose fermenters. Dulcite and saccharose fermentation varied and formed the basis of a classification into four main groups. Group I fermented dulcite but not saccharose, Group II neither dulcite nor saccharose, Group III both dulcite and saccharose, and Group IV saccharose but not dulcite.

Most dulcite-fermenting strains produced acid or acid and gas overnight, but many only did so after 48 hr. incubation and a few required 3–6 days' incubation. Acid production was usually temporary, however, alkalinity appearing 2–7 days later. Production of H_2S could not be detected. No strain liquefied gelatin. Some were Voges-Proskauer-positive, others negative; some methyl-red-positive, others negative. Most, but not all, produced indole.

A short discussion on the properties of each group follows (see Table 5).

Paracolon bacilli, Group I. Fifty strains were isolated, forty-eight from infants suffering from diarrhoea, one from a control and one from an otorrhoea associated with infantile diarrhoea. Thirty were late lactose-fermenters; ten produced acid without gas in mannite, the remainder acid and gas; six strains fermented dulcite in 24 hr. the remainder in 2–6 days. No strain was motile. Thirty-nine strains produced indole.

Paracolon bacilli, Group II. Thirteen strains came from infants suffering from diarrhoea, one from a normal infant. Ten were late lactose-fermenters. All strains fermented glucose, maltose and mannite. Nine produced abundant gas in these sugars, but the others only a tiny bubble in one or all. Eight strains produced indole and one was motile.

Paracolon bacilli, Group III. Thirty-three strains were isolated, twenty-seven from infants suffering from diarrhoea and six from normal infants. Five failed to ferment maltose, forming a maltose-negative subgroup. Glucose, mannite, and saccharose were fermented overnight with acid-gas production. Eighteen strains attacked dulcite after 2–4 days, the remainder in 24 hr. Ten were non-lactose fermenters, the remainder late-lactose fermenters. Twenty-six strains produced indole. Three strains were motile.

Paracolon bacilli, Group IV. Eleven strains were placed in this group, eight from infants suffering from diarrhoea, two from controls, and one from a milk specimen. All fermented glucose, maltose, mannite and saccharose, producing acid and gas in 24 hr. Lactose was not fermented by two strains and all the others fermented it late. Indole was produced by five strains. Three strains were motile. In seven strains the initial acidity in one or more of the sugar media was replaced by alkalinity 5–8 days later.

(2) *The investigation for stability or change in biochemical activity after subculture in artificial media*

Many bacteria which attack certain sugars when recently isolated fail to do so after subculture for some time in artificial media. On the other hand, some bacteria after subcultivation exhibit enzymes against carbohydrates which they did not attack when freshly isolated. The biochemical reactions and indole production of some paracolon strains were therefore retested 1–8 months after isolation.

Technique. Some of the growth in gelatin was emulsified in saline and spread on to a MacConkey or Hynes plate. Single colonly cultures were then investigated.

Paracolon bacilli, Groups I and III. Twenty-two strains were retested. No important changes were noted.

Paracolon bacilli, Group II. Five of the six strains re-examined remained unchanged. Strain 213(1), however, had now become a dulcite and saccharose fermenter.

Paracolon bacilli, Group IV. Of five strains tested, one failed to ferment saccharose, two others fermented dulcite.

B. SEROLOGICAL EXAMINATION OF PARACOLON BACILLI, GROUPS I–IV

Antisera were prepared in rabbits against eighteen strains. Four to six intravenous injections of carbol-saline-killed non-motile strains were given at 3–5 day intervals. The initial dose was 1000 million bacteria, followed by 2, 4, and 8 thousand million, and if necessary 8 and 16 thousand million. The rabbits were bled 4–6 days after the final injection. To every 9 c.c. of serum, 1 c.c. of 5 % phenol saline was added. High-titre antisera were generally easy to produce, but the titres varied from 1/500 to 1/20,000. Most were about 1/2500.

The stock bacterial suspensions were diluted with normal saline to an opacity of about 400 million *Bact. coli* per c.c. for the agglutination tests. The serum dilutions used were 1/25, 1/50, 1/125, 1/250, 1/500, etc., up to the final titre of the serum; later this series was often unnecessary, only the last four dilutions being used in many cases, e.g. if the full titre of the serum was 1 in 2500, the dilutions were 1/250 to 1/2500.

Paracolon bacilli, Group I. Sera were prepared against strains 42(1), 78(*a*), 145(1), 159(2), 205(2), 209(3) and 302(*y*).

Each serum was tested against all fifty strains. Table 6, which is a summary of a large number of experiments, shows that thirty-nine strains agglutinated to full titre with antisera 42(1), 145(1) and 205(2) and either failed to agglutinate or agglutinated to low titre only with the other antisera.

Strains 78(*a*), 209(3), 301(*y*), and 302(*y*) agglutinated to full titre with the antisera formed by the first, second and fourth of these strains and thus appeared to form a group.

Only two strains agglutinated fully with antiserum 159(2), though several agglutinated partially. Five inagglutinable strains remained.

perature. After centrifuging, the clear supernatant fluid was removed for use.

Experiments

Group I. Five series of absorption experiments were performed with the Group I sera and strains. Each serum was absorbed by its homologous strain and by

Table 5. *Showing the biochemical activities of the four groups of paracolon bacilli. All strains were Gram-negative rods, either non- or late-lactose fermenters and non-gelatin liquefiers*

Group	No. of strains	Glucose	Maltose	Mannite	Dulcite	Saccharose	Indole production	Motility
I	50	AG	AG	A or AG	AG or –/AG →Alk.	—	Usually + Occ. –	Nil
II	14	AG	AG	AG	—	—	+ or –	13 nil 1 pos.
III	28	AG	AG	AG	AG or –/AG →Alk.	AG	Usually + Occ. –	30 nil 3 pos.
Subgroup	5	AG	—	AG	–/AG→Alk.	AG		Nil
IV	11	AG Occ.→Alk.	AG Occ.→Alk.	AG Occ.→Alk.	—	AG Occ.→Alk.	+ or –	8 nil 3 pos.

AG = acid and gas.
A = acid.
– = acid and gas absent, or negative.
+ = positive.
→Alk. later becoming alkaline.
–/AG = acid and gas production after 24 hr.
Occ. = occasionally.

Table 6

Suspension	Antisera 42(1)	145(1)	205(2)	78(*a*)	209(3)	302(*y*)	159(2)
145(1)	1/5000	1/500	1/2500	Neg.	Neg.	Neg.	1/50
42(1)	1/5000	1/500	1/2500	1/25	1/50	Neg.	1/250
261(2) and 36 other strains	1/5000	1/500	1/2500	Neg.	1/25	Neg.	1/125
78(*a*)	1/500	1/100	1/50	1/2500	1/2500	1/20,000	Neg.
209(3)	1/250	1/50	Neg.	1/2500	1/2500	1/20,000	Neg.
301(*y*)	1/2500	1/100	1/500	1/2500	1/2500	1/20,000	Neg.
302(*y*)	1/500	1/100	1/500	1/2500	1/2500	1/20,000	Neg.
159(2)	1/50	1/50	1/50	1/250	1/250	Neg.	1/2500
376(1)	1/25	1/25	1/50	1/250	1/250	Neg.	1/2500
40	Neg.	1/50	1/50	Neg.	Neg.	Neg.	Neg.
122(1) and 3 other strains	1/50	Neg.	1/50	Neg.	Neg.	Neg.	Neg.

The titres given in this and subsequent tables were the last tubes in which complete or incomplete agglutination occurred; partial and trace agglutinations were not recorded for the sake of clarity.

Agglutinin absorption

Technique

A series of absorption of agglutinin experiments were next performed to test the serological relationship of these strains.

Very dense suspensions (about 50,000 million bacilli per c.c.) were prepared in 0·5 % carbol-saline, 4 c.c. of which were added to 1 c.c. of antiserum. The mixture was shaken and incubated at 37° C. for 2 hr. It was then centrifuged and the clear supernatant fluid removed. Sometimes this procedure sufficed, but often a second dose of suspension was added to the supernatant fluid, followed either by incubation for 2 hr. or standing overnight in the refrigerator or on the bench at room temother members of Group I, and the absorbed serum tested against the homologous, absorbing and ten other strains.

In Series A antiserum 42(1) was absorbed by suspensions 42(1), 145(1), 79(*a*) and 159(2), and it was found that the first two completely absorbed the agglutinins from this serum for all strains. Absorbing suspensions 78(*a*) and 159(2) failed to affect the titre of the serum for strains 42(1), 145(1), etc.

In Series B, antiserum 145(1) was absorbed by suspensions 145(1), 42(1), 173(*x*), 159(2) and 78(*a*), and it was shown that each of the first three strains completely removed the agglutinins for all strains tested, whilst the last two absorbed the minor agglutinins for themselves.

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Series C proved for serum 205(2) what series A and B did for sera 42(1) and 145(1) respectively.

In Series D, antiserum 78(*a*) was absorbed by strains 78(*a*), 209(3), 42(1), 145(1) and 159(2), and it was found that the first two completely absorbed this antiserum for themselves and strains 301(*y*) and 302(*y*), whilst strains 42(1), 145(1) and 159(2) were unable to do so, though capable of removing the weak agglutinins against themselves and related strains.

In Series E, antiserum 209(3) was absorbed by strains 209(3), 78(*a*), 42(1), 145(1) and 159(2), with results for serum 209(3) identical with those for serum 78(*a*) in Series D.

These results showed that thirty-nine out of the fifty strains had the same antigenic structure; that strains 78(*a*), 209(3), 301(*y*), and 302(*y*) were also identical but possessed common agglutinins with these thirty-nine strains, and that strains 159(2) and 376(1) were antigenically similar and possessed minor antigens in common with many of the other strains.

Further investigations showed that at least three major antigens were present in strains 78(*a*), 209(3), etc. One was that of strains 42(1), 145(1), 205(2), etc.; another was the antigen of strain 115(1), of Group III; and the third was the specific *Bact. alkalescens* factor. Another antigen may exist and be specific for these strains.

For discussion purposes, it is convenient to name these antigens as follows:

A = main antigen(s) of strains 42(1), 145(1) and thirty-seven others in Group I.

B = type-specific antigen of strains 78(*a*), 209(3), 301(*y*) and 302(*y*) in Group I.

C = antigen(s) of strain 115(1) and seven others of Group III.

Alk. = specific *Bact. alkalescens* antigen.

Group II. Antisera against strains 50, 70, 213(1), 289(*y*) and 360(*z*) were tested against all members of the group. Excluding strains 70 and C 54, no cross-agglutination occurred, indicating that most Group II strains are not serologically related. Strains 70 and C 54* went to full titre with serum 70.

Cross-agglutination and absorption experiments showed that these organisms possessed the A antigen of Group I strains to full titre, but also possessed as a minor component the C antigen of many Group III strains.

Further experiments showed that strain 213(1) also possessed the A antigen of strain 145(1) (Group I), the D antigen of strain 77(*a*) (Group III), and a specific antigen of its own.

Strain 360(*z*) was found to contain the C antigen of Group III and a type-specific factor.

Group III. Sera were prepared against strains 77(*a*), 115(1), 237(1), 319(*x*), 256(*x*) and 247(*x*).

Cross-agglutination experiments divided the strains into four groups. Nine strains agglutinated to full titre with sera 115(1) and 319(*x*), three strains with sera 77(*a*) and 237(1), thirteen with serum 247(*x*), and the remaining eight strains failed to agglutinate with any antisera. However, sera 77(*a*) and 237(1) agglutinated to lower titre those strains going to full titre with sera 115(1) and 319(*x*); moreover, the latter contained minor agglutinins for strains 77(*a*), 237(1) and 271(*x*). Series of absorption experiments were carried out, each absorbed serum being tried against selected members of the group (Table 7).

Table 7. *Series A. Showing the results of the agglutinin-absorption experiments*

	Antiserum 42(1)				
Suspension	Unabsorbed titre	Absorbed by 42(1) titre	Absorbed by 145(1) titre	Absorbed by 78(*a*) titre	Absorbed by 159(2) titre
42(1)	1/5000	Neg.	1/25	1/5000	1/5000
205(2)	1/5000	Neg.	Neg.	1/5000	1/5000
334(2)	1/5000	Neg.	Neg.	1/5000	1/5000
145(1)	1/5000	Neg.	Neg.	1/5000	1/5000
78(*a*)	1/500	1/500	1/50	Neg.	1/500
209(3)	1/250	1/250	1/50	Neg.	1/250
159(2)	1/50	Neg.	Neg.	Neg.	Neg.

In Series I: Serum 115(1) was absorbed by suspensions 115(1), 323(*y*), 77(*a*) and 237(1). The first two completely absorbed this serum for all strains tested, but neither strain 77(*a*) nor 237(1) did so.

In Series II: Serum 319(*x*) was absorbed by suspensions 319(*x*), 115(1), 77(*a*) and 237(1), proving for this serum what series I did for serum 115(1).

In Series III: Serum 77(*a*) was absorbed by suspensions 77(*a*), 237(1) and 115(1).

The first two strains completely absorbed both the major agglutinins for themselves and strain 271(*x*) and the minor agglutinins for strains 115(1), but strain 115(1) absorbed only the minor agglutinins for itself and two others, without lowering the titre for strains 77(*a*) and 237(1).

In Series IV: Serum 237(1) was absorbed by suspensions 237(1), 77(*s*) and 115(1). The results were similar to those in series III except that absorbing strain 115(1) reduced the titre of the serum for strains 77(*a*) and 271(*x*).

The results of these experiments showed that two important antigens existed in these twelve strains. One of these, antigen C, was the major antigen in nine (115(1), 319(*x*), etc.), and the other, antigen D, in three strains (77(*a*), 237(1), and 271(*x*)). The members of each subgroup were serologically identical. Each

* This strain was motile. Boiled suspensions of this and the other motile strains were used.

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subgroup, moreover, possessed a small amount of the other's major antigen.

Thirteen strains agglutinated to full titre with serum 247(*x*). This serum was absorbed separately by the homologous strain and two others, and then contained no agglutinins for any of the thirteen suspensions. All these strains were probably identical, although complete 'mirror tests' would be required to prove this conclusively. This main antigen may be called antigen E.

Cross-agglutination experiments between the Group III suspensions and anti-A sera of Group I gave positive results in these thirteen cases. In general the titres obtained were high, some going to full titre; antiserum 247(*x*), however, failed to agglutinate any Group I Type A strains.

C. Antigenic relationship of the paracolon bacilli

(1) *The dysentery bacilli*

Smith (1941) showed that a Flexner dysentery bacillus and a paracolon bacillus isolated from the same faeces were serologically identical. Bamforth (1936) described twenty-one paracolon strains as serologically identical with *Bact. alkalescens*.

In this investigation the relationship was investigated by agglutination and agglutinin absorption technique. The anti-dysentery sera were those of the Standards Laboratory, Oxford, except for the anti-*alkalescens* serum which was prepared in a rabbit* (see Part I). The dysentery strains obtained from the National Collection of Type Cultures were Sonne (no. 2182), Shiga (no. 4837), Flexner V (no. 4832), Flexner W (no. 4833), Flexner X (no. 4834), Flexner Z (no. 4835), Newcastle (Denton I) (no. 4720), Newcastle (Newcastle I) (no. 3083), *alkalescens* (Towne) and *ambiguum* (no. 306).

Each strain was tested against the homologous Oxford antiserum. All except the Flexner Y strain agglutinated to full titre.

Paracolon bacilli, Group I. Twenty-three strains were tested against the dysentery antisera and all, as shown in Table 9, were agglutinated by one or more sera.

When the Group I antisera were tested against dysentery suspensions, low-titre agglutination or a negative result was found, except with *Bact. alkalescens*.

Table 8. *Series I. Showing the result of absorption experiments in Group III*

	Antiserum 115(1)				
Suspension	Unabsorbed titre	Absorbed by 115(1) titre	Absorbed by 323(*y*) titre	Absorbed by 77(*a*) titre	Absorbed by 237(1) titre
34	1/2500	1/25	Neg.	1/2500	1/2500
115(1)	1/2500	Neg.	Neg.	1/2500	1/2500
254(*b*)	1/2500	1/25	Neg.	1/2500	1/2500
13(1)	1/2500	1/25	Neg.	1/2500	1/2500
319(*x*)	1/2500	Neg.	Neg.	1/2500	1/2500
323(*y*)	1/2500	1/25	Neg.	1/2500	1/2500
77(*a*)	1/50	Neg.	Neg.	Neg.	Neg.
237(1)	1/50	Neg.	Neg.	Neg.	Neg.

Series III

	Antiserum 77(*a*)			
Suspension	Unabsorbed titre	Absorbed by 77(*a*) titre	Absorbed by 237(1) titre	Absorbed by 115(1) titre
77(*a*)	1/500	Neg.	Neg.	1/500
237(1)	1/500	Neg.	Neg.	1/1000
271(*x*)	1/500	Neg.	Neg.	1/500
115(1)	1/50	Neg.	Neg.	Neg.
34	1/100	Neg.	Neg.	Neg.
323(*y*)	1/50	Neg.	Neg.	Neg.

Absorption of sera 42(1) and 205(2) (anti-A) by strains 247(*x*) and 390(2) showed that they possessed the A antigen in large amount.

Absorption of serum 247(*x*) by strains 145(1), 42(1) and 173(*y*) proved that the E antigen of strain 247(*x*) was not possessed by type A strains.

Group IV. Antisera 153(1) and 324(1) were prepared and tested against the members of the group. Only very small titres were recorded.

Cross-agglutination experiments with the other groups followed by absorption tests showed that strain 324(1) was identical with strain 115(1) (Type Cd) of Group III. Finally, strain 362(*x*) went almost to full titre with serum 42(1) (anti-A), and strain 309(*x*) agglutinated fully to titre with serum 289(*y*) Group II.

The diagrams in Fig. 1 are attempts to represent the antigenic structure of various paracolon bacilli.

* High titre anti-V and anti-W Flexner sera, prepared in the laboratory, were used for absorption experiments.

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(2) Bact. alkalescens

Three sera went to full titre against *Bact. alkalescens*, namely, 78(*a*), 209(3), and 302(*y*). Moreover, these strains and strain 301(*y*) were agglutinated to full titre by the anti-*alkalescens* serum. 'Mirror' tests showed that the *alkalescens* antigen was a major component in 78(*a*), 145(1), 159(2) and 173(*y*), and although the agglutinins against these strains were removed, the titres for the Flexner strains were full or partially reduced.

Further absorption experiments were performed using high-titre anti-Flexner sera.

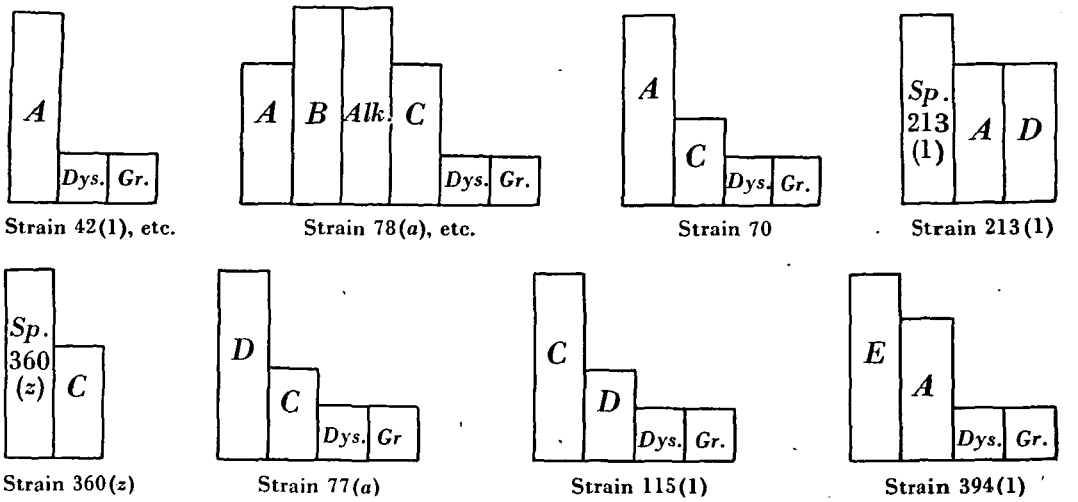

Fig. 1. *A*, specific antigenic component of strain 42(1) and others of Group I; also present in sixteen other strains. *B*, ? specific antigen strain of 78(*a*) and three others. *C*, major antigen of strain 115(1) and seven other strains of Group III. *D*, major antigen of strain 77(*a*) and two other strains of Group III. *Alk.* specific antigen of *Bact. alkalescens*. *Dys.* various antigenic components of the other dysentery bacilli. *Gr.* group minor antigens common to various strains of paracolon bacilli. *E*, specific antigen of thirteen members of Group III which contain the A component. *Sp.* specific antigen.

Table 9. *Showing the number of strains of paracolon bacilli, Group I, tested against the dysentery antisera, the numbers agglutinating and the titres found*

Oxford sera (full titre 1/250)	Titre 1/250	Titre 1/125–1/50	Titre 1/25	Negative or trace	Total strains examined
Newcastle	1	7	2	12	22
Flexner Group I (anti-V, W, X, Y and Z)	0	12	5	3	20
Flexner V	0	0	2	18	20
W	0	3	6	10	19
X	0	4	5	14	23
Y	0	0	2	18	20
Z	0	0	2	7	9
Sonne	0	2	6	14	22
Shiga	0	0	1	22	23
	1/2500 or 1/1250				
alkalescens (titre 1/2500)	4			17	21

these organisms, but that other antigens were present (see Fig. 1).

(3) Bact. dysenteriae *Flexner*

Twenty out of twenty-three strains tested agglutinated strongly with one or more Flexner sera. A preliminary 'blunderbuss' absorption experiment was performed. The Flexner Group I antiserum (titre 1/250) was absorbed simultaneously by suspensions 42(1), The results obtained with strains 42(1), 'Clarke', 145(1), 261(2), etc., individually with anti-V and anti-W sera proved that the antigens were minor and easily absorbed factors.

(4) Bact. dysenteriae *Newcastle*

Eight of twenty-two strains tested agglutinated strongly (one to full titre) with the anti-Newcastle

serum, but no Group I serum contained agglutinins for the Newcastle bacillus. These results suggest that a common antigen frequently exists between the Newcastle bacillus and these strains, but that each is type specific. Two absorption tests using strains 42(1) and 173(*y*) and the Newcastle antiserum confirmed this view.

(5) Bact. dysenteriae *Sonne*

Eight of twenty-two strains tested gave strong agglutination with the Sonne antiserum, but only two paracolon sera contained agglutinins for Sonne's bacillus and these to low titre. Absorption experiments proved that the common antigens were minor ones and easily absorbed.

(6) Bact. dysenteriae *Shiga*

Only one strain and one serum of those tested gave agglutination.

The titres recorded using the repeatedly subcultured organism were always lower than in the initial experiments and often negative.

Paracolon bacilli, Group II. Eight out of ten strains tested against the dysentery antisera gave no agglutination. Strain 70 (Type Ac) agglutinated almost to titre with both the Flexner Group I and the Flexner X sera. Strain 50 went to full titre with the Shiga antiserum, but the Shiga suspension was inagglutinable by antiserum 50. The full agglutination occurred with strain 50 when freshly isolated, but agglutination was negative with suspensions made from subcultures months later. The original culture was shown to be unmixed with Shiga's bacillus. It seems therefore that strain 50 lost its Shiga antigen quickly in subculture.

Antisera 50 and 213 produced no agglutination with the dysentery bacilli, but 289(*y*) (titre 1/1000) agglutinated Flexner W and X to a titre of 1/50 and *Bact. alkalescens* to a titre of 1/125.

Paracolon bacilli, Group III. Twenty-two members were tested, including six 'unclassified' strains. These were all negative against the dysentery sera. Type Dc strains were also negative, but serum 237(1) gave low-titre agglutination with *Bact. alkalescens*. All Type Cd and Ea strains tested agglutinated with one or more of the Flexner sera or with the Sonne or *alkalescens* sera usually to low or intermediate titres. No agglutination occurred with Shiga or Newcastle antisera.

Antiserum 115(1) produced a high titre for *Bact. alkalescens*, but serum 324(*x*) a low titre for both this organism and the Newcastle bacillus.

Two absorption tests, using high-titre anti-*alkalescens* and anti-Flexner W sera, showed that strain 115(1) absorbed the agglutinins from these sera without affecting the titres for the homologous strains.

Paracolon bacilli, Group IV. Eight strains were examined against the dysentery sera. Five were completely negative. Strain 324(1) (Type Cd) produced a low titre against the Flexner Group I antiserum; strain 309(*x*), containing some antigen A, gave a high titre with anti-Shiga serum; the unclassifiable strain 244(*x*) went to full titre with the Flexner Group I serum and almost to full with the anti-W and anti-Y sera. These results were obtained when the organisms were freshly isolated, but agglutination was absent when suspensions made several months later were used.

From these experiments the position was fairly clear. Four Group I strains contained the *alkalescens* antigen to full titre and as a 'permanent' component. The relationship of Group I strains to other dysentery bacilli was less striking. Various common, minor antigens were usual between them and the Flexner and Newcastle bacilli in particular. When freshly isolated, nearly all the paracolon bacilli included in the A, B, C, D, E classification contained minor antigens in common with various members of the dysentery group. After repeated subculture these minor antigens either diminished in quantity or disappeared. Some others, not included in the A, B, C, D, E mosaic, also possessed minor components in common with the dysentery bacilli, but most of these were Group I strains. In fact, one of these went to full titre with the Newcastle serum. Most of the Groups II, III, and IV strains, which do not possess the A, B, C, D E antigens, had no antigens in common with the dysentery bacilli.

D. Agglutination experiments with Salmonella antisera

Sixty-four strains, thirty belonging to Group I, eight to Group II, twenty to Group III, and six to Group IV, were tested against the Oxford Polyvalent Salmonella antiserum (titre 1/250). Fourteen strains were tested against the Oxford anti-typhosum O, anti-paratyphosum AO, anti-aertrycke O and anti-paratyphosum CO sera. All the tests gave negative results.

E. Agglutination experiments using strains of *Bact. morgani* and paracolon antisera

Boiled suspensions of six strains of *Bact. morgani*, four from cases and two from controls, were tested against the paracolon antisera. All the results were negative.

F. Agglutination experiments using *coli-aerogenes* strains against the paracolon and dysentery antisera

Seven *coli-aerogenes* strains, four isolated from normal infants, and three stock cultures from the Department of Bacteriology, Trinity College, Dublin, were used. Biochemically, three were dulcite +, saccharose + strains, three dulcite +, saccharose −, and one dulcite −, saccharose −. One of the stock cultures was a 'true *Bact. coli*'.

Boiled suspensions were tested against the paracolon antisera, with negative results.

The seven strains were tried against the dysentery antisera. One strain gave a titre of 1/25 with Flexner Group I serum, and the remainder negative results.

G. Relationship of *B. asiaticum*, *B. columbense* and B 5659 (Dudgeon Type A) to the paracolon strains isolated

Cultures of *B. asiaticum* (strains 1747 and 6169), *B. columbense* (Castellani) (708) were received from the Lister Institute, and B 5659 (Dudgeon Type A) was obtained from St Thomas's Hospital, London, through the kindness of Dr Bamforth.

It was found that biochemically B 5659 (Dudgeon) and *B. columbense* belonged to the Group I paracolon

bacilli, whilst the *B. asiaticum* strains belonged to Group IV. As received, these strains were non-motile.

Boiled suspensions of these organisms were used in the agglutination tests to destroy remnants of the flagellar substances and each suspension was tested against the paracolon antisera.

B. asiaticum and *B. columbense* failed to agglutinate with any of the antisera, but B 5659 (Dudgeon) went to full titre against and completely absorbed the agglutinins from antisera 42(1) and 205(2)—(anti-A). In the absence of a complete 'mirror' it may be tentatively concluded that B 5659 (Dudgeon) is a member of the Group I Type A strains.

No agglutination was found with these organisms against the dysentery antisera.

H. THE PARACOLON BACILLI ISOLATED FROM THE SERIES OF 100 NON-CONTACT CONTROLS

The paracolon bacilli isolated from the controls have been included in the previous pages, but a separate summary follows. Eleven strains were isolated, one Group I Type A, one Group II Type Ac, six Group III (3 Ea, 1 Cd, in type) and three Group IV. They differed in their characteristics in no way from the strains isolated from the cases except that six were motile. These were the three Group IV strains, two Group III strains, and the Group II strain.

All strains were tested against the Oxford dysentery antisera. In general they behaved much as the strains isolated from the cases, but with lower agglutination titres.

SUMMARY

The Group I paracolon bacilli formed biochemically a stable group. They fermented glucose, maltose, mannite, and dulcite, but not saccharose. Most of them could be distinguished from Salmonella organisms by the late fermentation of lactose or the production of indole. Many resembled the Newcastle bacillus in being late-dulcite fermenters. Serologically 75 % of the strains were identical, each containing the A antigen. Some formed a separate group containing the A antigen, and *Bact. alkalescens* antigen and the C antigen of some Group III strains. The A strains are probably identical with those of Dudgeon & Pulvertaft (1927), but their strains were described as being motile. All Group I strains had minor agglutinins in common with the dysentery bacilli, particularly when freshly isolated. Most of Dudgeon's strains were isolated from cases of urinary infection, but in the present investigation almost all were isolated from the discharges of infants with diarrhoea and enteritis. This organism may be regarded as pathogenic or facultatively so, and deserves a name. I suggest it would be appropriate to attach to it the name of Dudgeon, who was the first to call attention to it.

Biochemically Group III bacilli resembled Group I strains but differed in fermenting saccharose. Some were motile. The metabolic and biochemical properties examined were stable. Serologically many (40 %) possessed the A antigen, but also possessed a type-specific antigen, E. Others (37 %) possessed both C and D antigens, usually C but sometimes D was dominant. These strains are related to the dysentery bacilli by the possession of common, minor antigens. A group of serologically heterogeneous strains remained (23 %), generally unrelated to the dysentery bacilli.

Most Group II bacilli were stable biochemically, but some were not. Many strains of Group IV were unstable. Serologically, Groups II and IV were mostly heterogeneous, although 24 % were included in the A, B, C, D, E pattern. Together they formed but a minority of all the paracolon bacilli.

Further serological investigation is required to discover new antigens and to link up the paracolon bacilli isolated from various sources.

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