Non-toxigenic clostridia in babies

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SUMMARY Non-toxigenic clostridia, *Clostridium butyricum* but occasionally *C. paraputrificum*, were isolated from mainly extra-alimentary sites of 28 babies in a maternity hospital over a period of 40 weeks. During that time, *C. butyricum* was isolated from the blood of two babies who died of necrotising enterocolitis and of two babies without referable symptoms. Otherwise these organisms appeared to be of no pathogenic significance.

In a recent outbreak of necrotising enterocolitis (NEC),¹ *Clostridium butyricum* was isolated from the blood or stools of the majority of affected babies and was considered to be a primary cause of the disease, although this conclusion was subsequently challenged.^{2,3} During 1977 we isolated a similar *Clostridium* species from several babies in the John Radcliffe Maternity Hospital, Oxford, both from blood cultures and from extra-alimentary sites. Only occasional isolates were recorded before and after this period. Initially, the isolates were identified as *C. paraputrificum*, but subsequently most were reclassified as *C. butyricum*. We report here our clinical and bacteriological findings.

Methods

Bacteriological specimens were submitted regularly from all babies in the special care unit; babies in the general wards were swabbed when necessary for minor septic lesions. Later in the survey, rectal swabs were cultured whenever clostridia were grown from other sites. Specimens were cultured by routine laboratory methods, both aerobically and anaerobically, with cooked meat broth as the only enrichment medium. Some direct inoculations and broth subcultures were made on to horse blood agar containing kanamycin, 100 mg/l, incubated anaerobically for 48 hours in an anaerobic jar with a gas mixture of 90% hydrogen and 10% CO₂. Clostridia were identified by:

- 1 A routine laboratory 'sugar set', including glucose, maltose, lactose, sucrose, and mannitol peptone waters, and tests for indole production, nitrate reduction, gelatine liquefaction, and lecithinase. Sporulation was tested on egg yolk agar incubated at 37°C for two to five days.

- 2 API 20^A test strips (API System SA). These incorporate additional carbohydrate substrates including xylose, arabinose, and raffinose.
- 3 Several strains were investigated by the API-ZYM method (API System SA).
- 4 Thirty-one strains from 25 babies were finally submitted to Dr AT Willis, Public Health Laboratory, Luton, who confirmed the identification of all strains.

Results

A *Clostridium* sp (*butyricum* or *paraputrificum*) was isolated from 28 babies over a period of 40 weeks. The clinical and bacteriological findings are summarised in the Table. Five of the babies are considered in more detail here.

CASE 1

A female infant of 36 weeks' maturity and weighing 1913 g was delivered by Caesarean section because of tachycardia after amniocentesis. The baby was asphyxiated at birth but responded to resuscitative measures and blood transfusion for anaemia. Progress was satisfactory until 4 days of age when she became pale, shocked, and lethargic, vomited, and passed bloody stools. Abdominal x-rays showed gas in the bowel wall but no signs of perforation. Despite treatment with intravenous fluids, blood transfusion, antibiotics, and ventilatory support, her condition rapidly deteriorated, and she died 20 hours after the onset of symptoms. *C. butyricum* was isolated from a gastric aspirate and from blood taken during the terminal illness. Bottle feeds (SMA) were given in addition to breast milk.

CASE 2

A male infant weighing 2180 g was delivered vaginally

Isolation of C. butyricum and C. paraputrificum from neonates: clinical and bacteriological details

Infant	Birth weight (g)	Gestation (wk)	Source	Age (days)	Clostridium sp.	Feed	Clinical diagnosis
1	1913	36	Blood	4	B	EBM Bottle	See text
			Gastric aspirate	5	B		
2	2180	32	Abdominal wound	16	B		See text
			Rectum	16			
			Blood	32			
3	1210	28	Umbilicus	47	B	EBM	See text
			Blood	47	B		
4	1530	33	Blood	4	B	EBM	See text
5	1620	34	Wound	33	B	EBM	See text
			Umbilicus	36			
			Rectum	36			
			Umbilicus	43			
6	3065	39	Umbilicus	1	B	Bottle	Mild asphyxia, pyrexia
7	1400	30	Rectum	45	B	EBM	Prematurity, asphyxia
							hyaline membrane
8	2780	36	Eye	6	B	Bottle	Preterm, asphyxia, jaundice
			Rectum	7	B		
			Umbilicus	7	B		
9	1520	31	Umbilicus		B	EBM	Prematurity, asphyxia
10	1640	33	Umbilicus	8	B	EBM	Respiratory distress, jaundice, inflamed umbilicus
11	2980	38	Umbilicus	12	B	EBM Bottle	Asphyxia, tachypnoea hypoglycaemia, convulsions, jaundice, pyrexia
12	2150	38	Eye	4	B	Bottle	Sticky eye, loose stools
13	1690	34	Rectum	2	B	EBM	Prematurity, cyanosis, jaundice
14	1680	32	Umbilicus	25	P	EBM Bottle	Prematurity, hyaline membrane
15	3990	40	Umbilicus	3	P	Bottle	Cyanosis, stridor
16	2600	35	Umbilicus	7	B	Bottle	Preterm, respiratory distress, ?septicaemia
17	2900	38	Umbilicus	3	B	Bottle	Asphyxia, hypothermia, hypoglycaemia
18	2280	35	Umbilicus	13	B	Breast Bottle	Preterm, drug depression
19	3015	39	Umbilicus	15		Bottle	Asphyxia, hypothermia
20	3245	40	Umbilicus	7	P	Breast Bottle	Umbilical hernia, jaundice
21	3620	38	Umbilicus	2	B	Bottle	Sticky eye, pyrexia
22	2890	40+	Umbilicus	13	B	Breast Bottle	Jaundice
23	3870	40	Umbilicus	6	P	Breast Bottle	Jaundice
24	3150	40	Umbilicus	7	B	Breast	Jaundice
25	2540	36	Eye	9	B	Breast Bottle	Jaundice, sticky eye
26	2980	40	Umbilicus	8	B	Bottle	Moist cord
27	2615	37	Umbilicus	3	B	Breast	Asphyxia, jaundice
28	3980	41+	Groin	9	B	Breast	Jaundice
			Umbilicus	9	B	Bottle	

EBM—expressed breast milk.

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at another hospital at 32 weeks' gestation, four weeks after membrane rupture. Progress during the first four days was good. On the fifth day he developed apnoeic attacks, a distended abdomen, and bloody stools. X-rays showed free gas in the abdomen. Initially he was treated with ventilatory support, plasma, and antibiotics (penicillin and gentamicin). Laparotomy on the eighth day disclosed a perforation of the transverse colon, and a colostomy was fashioned. A blood culture taken at this time grew *Escherichia coli*, and a peritoneal swab grew *Esch. coli* and *C. perfringens* (*C. welchii*). At 16 days the infant's condition was complicated by electrolyte loss, wound breakdown, faecal fistula, and free gas in the peritoneum. *C. butyricum* was isolated from the abdominal wound and faeces and subsequently from a blood culture taken at 32 days. Complications proved insuperable and the child died at 41 days.

CASE 3

A male twin infant was delivered at 28 weeks' gestation and subsequently developed hyaline membrane disease which required ventilatory support. An umbilical artery catheter was inserted, and he received an exchange transfusion for deepening jaundice. At 7 weeks cultures were obtained because of apnoeic attacks and bradycardia. *C. butyricum* was isolated from blood and from an umbilical swab. These isolates were regarded as contaminants. His condition slowly improved and he was discharged home at 8 weeks. He had received expressed breast milk (EBM) only.

CASE 4

A female infant weighing 1530 g at birth was delivered by Caesarean section at 33 weeks' gestation because of maternal pre-eclampsia. The baby was asphyxiated at birth and required intubation. Subsequently she did well until the fourth day when cultures were taken because of hyperirritability with a blood sugar of 1.1 mmol/l. *C. butyricum* was isolated from blood culture but was discounted as the baby recovered without antibiotic therapy. She received EBM only.

CASE 5

A male infant weighing 1620 g was delivered by forceps at 34 weeks' gestation. After transient hyaline membrane disease he developed tachypnoea and oxygen-dependence, ascribed to fibrosing alveolitis. CO₂ was infused into the peritoneal cavity to outline the diaphragm. The entry wound became infected, and a swab of the lesion grew *C. butyricum*. The same organism was also isolated from umbilical and rectal swabs but at no time

caused any attributable symptoms. He received EBM only.

C. butyricum was isolated from sweep plate cultures taken from the cot blankets of two babies, one of whom was known to be colonised with the organism. No further environmental sampling was carried out, and the attendants in the unit were not investigated for possible carriage of the organism. No similar isolations were made from other cultures being processed in the laboratory during the same period. The antibiotic aerosol (Framispray) in use at that time for the routine disinfection of cords was sprayed on to blood agar plates and into cooked meat medium, but cultures were sterile. Twenty isolates of *Clostridium* spp. from 16 infants were tested for sensitivity to bacitracin, a constituent of the spray, and all were found sensitive using bacitracin 8 unit discs (Mast).

Discussion

Case 1 in our series was a low-birth-weight infant asphyxiated at birth who required resuscitation and developed NEC, *C. butyricum* appearing simultaneously in the blood. Case 2 was a low-birth-weight infant who developed NEC without any other predisposing factors. *C. butyricum* was not isolated from the blood until 27 days later during the terminal stages of the illness, and its importance is doubtful. Cases 3 and 4 did not develop NEC, although several possible predisposing factors were present, and *C. butyricum* isolated from blood culture was dismissed as a contaminant. In case 5, abdominal wound infection was acquired either by penetration of the bowel or from the skin. In 23 additional infants *C. butyricum* or *paraputrificum* was isolated from superficial sites without causing constitutional upset. The presence of these organisms could not be correlated with the type of feed, as it was not always indicated whether breast milk was fresh, pasteurised, or boiled.

C. butyricum has been isolated from normal appendix flora⁴ and from intra-abdominal wounds⁵ but was not generally considered to cause disease in man or animals until incriminated in the pathogenesis of NEC.¹ In a cluster of 12 cases occurring within six weeks at the Royal Free Hospital, *C. butyricum* was isolated from the blood of seven babies and from the stools of two others but was absent from the stools of 34 normal babies. Since the commonly accepted predisposing conditions for NEC were absent from the group, it was considered that *C. butyricum* was a primary invader with a pathogenic role in the late stages of the disease. Gas liquid chromatography (GLC) was used to identify the isolated strains and also to detect the presence of the organism in blood

and stools. However, *C. butyricum* gives rise to a GLC tracing indistinguishable from that of several other clostridial species.⁶ Other workers have found *C. butyricum* to be commonly present in the stools of infants unaffected by NEC.⁷ In a report of 17 cases of NEC from New Mexico,⁸ *C. butyricum* was isolated from the blood of one infant before death, but clinical severity appeared to be related more to the isolation of *C. perfringens* from the peritoneal fluid or blood of several patients. The clinical importance of bacteraemia due to non-toxicogenic clostridia is difficult to assess. It has been recognised as a clinical entity, characterised by fever, leucocytosis, and sometimes hypotension, indicating a non-specific response to the organism with a good prognosis.⁹ It is commonest in the newborn, in whom anaerobic bacteraemia in general is often self-limiting and rarely fatal.¹⁰ Clostridia may also occur as contaminants in blood cultures,¹¹ and it is perhaps significant that we isolated *C. butyricum* from the cot blankets of two babies known to be colonised with the organism.

In the present study, the differentiation of *C. butyricum* from *C. paraputrificum* by using a series of biochemical tests was rarely clearcut, and great reliance was placed on the spore position (*C. paraputrificum*-terminal; *C. butyricum*-subterminal). Consistent differences between strains so identified were detected by the API-ZYM method, and this will be the subject of a further report. A full description of *C. paraputrificum* was given by Hall and Snyder,¹² who isolated it from the faeces of infants during the first 10 days of life. It grew optimally at pH 6.9-8.2 and disappeared when the normal breast milk stool was established, the higher acidity pH 5.0-5.8 favouring overgrowth with bifidobacteria. These findings have been amplified in more recent work on the effect of different types of infant feeds on the bowel flora.¹³

In the present study, *C. paraputrificum* was isolated from the umbilical swabs of four babies, probably as the result of faecal contamination.¹² *C. paraputrificum* has occasionally been reported as a pathogen when isolated from blood, as in a 2-year-old infant with *C. paraputrificum* omphalitis and bacteraemia who subsequently died.¹¹ In a five-year review of clostridial bacteraemia in the USA,¹⁴ *C. paraputrificum* was considered significant in only two patients, including an elderly woman with decubitus ulcers, from whom multiple blood cultures yielded the organism before death. *C. paraputrificum* has also been reported in the blood of a diabetic patient with gas in the portal vein after mesenteric artery thrombosis.¹⁵

There is no firm evidence from our own and previous observations that *C. butyricum* or *para-*

putrificum can cause neonatal NEC, although it is likely that the disease results in a secondary bacteraemia. Indeed, it seems improbable that organisms lacking known toxins could give rise to a severe constitutional upset, and the possibility also exists that blood cultures may be contaminated with clostridia present on the skin or in the environment.

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References

- Howard FM, Flynn DM, Bradley JM, Noone P, Szawatkowski M. Outbreak of necrotising enterocolitis caused by *Clostridium butyricum*. *Lancet* 1977;2:1099-102.
- Gothefors L, Blenkharn I. *Clostridium butyricum* and necrotising enterocolitis. *Lancet* 1978;1:52-3.
- Kelsey MC, Vince AJ. Clostridia in neonatal faeces. *Lancet* 1979;2:100.
- Löhr W, Rabfeld L. *Die Bakteriologie der Wurmfortsatzenzündung und der appendikulären Peritonitis*. Stuttgart: Thieme, 1931.
- Gorbach SL, Thadepalli H, Norsen J. Isolation of 35 Clostridia species from clinical sources. Abstr 12th Interscience Conf. Antimicrob Agents Chemother 1972: 186.
- Holderman LV, Moore WEC, eds. *Anaerobic Laboratory Manual*. Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 1975.
- Laverdière M, Robert A, Chicoine R, Sulet D, Rosenfeld R. Clostridia in necrotising enterocolitis. *Lancet* 1978;2: 377.
- Kosloske AM, Ulrich JA, Hoffmann H. Fulminant necrotising enterocolitis associated with clostridia. *Lancet* 1978;2:1014-6.
- Alpern RJ, Dowell VR. Non-histotoxic clostridial bacteraemia. *Am J Clin Pathol* 1971;55:717-22.
- Chow AW, Leake RD, Yamanchi T, Anthony BF, Guze LB. The significance of anaerobes in neonatal bacteraemia: analysis of 23 cases and review of the literature. *Pediatrics* 1974;54:736-45.
- Wilson WR, Martin WJ, Wilkowske CJ, Washington JA. Anaerobic bacteraemia. *Mayo Clin Proc* 1972;47: 639-46.
- Hall IC, Snyder ML. Isolation of an obligately anaerobic bacillus from the feces of newborn infants and from other human sources and its probable identity with Köpfchenbakterien of Escherich, Rodella's *Bacillus* III, and *Bacillus paraputrificus* (Bienstock). *J Bact* 1934;28: 181-98.
- Bullen CL, Tearle PV, Willis AT. Bifidobacteria in the intestinal tract of infants: an in-vivo study. *J Med Microbiol* 1976;9:325-32.
- Rathbun HK. Clostridial bacteraemia without hemolysis. *Arch Intern Med* 1968;122:496-501.
- Wiot JF, Felson B. Gas in the portal venous system. *AJR* 1961;860:920.

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