

THE ASSOCIATION OF CERTAIN TYPES ( $\alpha$  AND  $\beta$ ) OF *BACT. COLI*  
WITH INFANTILE GASTRO-ENTERITIS

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Bray (1945), Giles & Sangster (1948), Giles, Sangster & Smith (1949), Taylor, Powell & Wright (1949) have called attention to the association of a particular variety of coliform bacillus with cases of gastro-enteritis. The organism has been variously named by Bray var. *Neapolitanum*, by Taylor, Powell and Wright D433, and in this laboratory, type  $\alpha$ , but under these various names it has the same cultural and antigenic characteristics. The antigenic specificity of this organism is established, and its distinction from other coliforms has seldom been difficult. One rather rare sub-type, however, has apparently some of the same serological characteristics (Taylor *et al.* 1949), though culturally it is anaerogenic and motile and, therefore, should have at least one antigen additional to those of the main type which is non-motile. In the previous paper (Giles *et al.* 1949) it was shown that in 1947 the  $\alpha$  type was associated with 94.7% of 207 cases of gastro-enteritis. The association of the organism with the disease was further indicated by its disappearance from the stools of 74 cases out of 84 examined at 4- to 5-day intervals when clinical improvement occurred. In the remaining 10, the organism was still present in the stools when the patient was discharged from hospital. In infants suffering from what appeared clinically to be non-infectious diarrhoea the  $\alpha$  organism was isolated from 1.5% of cases. Further, of specimens from 271 infants under the age of 2 years without diarrhoea, and a group of 450 adults and older children, only six showed the presence of the  $\alpha$  organism, and most of these had been in close association with cases of gastro-enteritis. Similar results have been reported by the other workers quoted above.

In November 1947, it was noted that the  $\alpha$  type was less frequently found in specimens from clinical cases of infantile gastro-enteritis. Other coliform organisms were obtained from definite cases and antisera were prepared for their 'O', 'K' and 'H' antigens (Kauffmann, 1947). By this means another fairly frequently occurring type was identified, and has been named for convenience the  $\beta$  organism. Up to the end of 1947 the  $\beta$  type was found in 21 cases of infantile diarrhoea of all kinds, in the specimens from three out of 53 healthy babies, and in 1 out of 74 adult controls. The bacteriological investigation

of cases of infantile diarrhoea was therefore continued during 1948, with results presented in this paper.

## TECHNIQUE

In this hospital all cases diagnosed by practitioners as gastro-enteritis are admitted to a ward which has eleven cubicles, and two rooms which can take four cots each with ample spacing. All cases on admission are isolated, and if it is decided that they do not suffer from infantile gastro-enteritis they can, if necessary, be transferred to the larger rooms, while those with definite gastro-enteritis are retained in the cubicle section. All possible precautions are taken against cross-infection. The equipment for sterilization, for the collection of soiled garments, and for nursing is in every way adequate, the only real difficulty being that of providing enough staff. The cases are under the care of the Professor of Child Health and his assistants.

As each case was admitted to the ward, a specimen of faeces was collected from the napkin (previously sterilized) and sent as soon as possible to the Laboratory for examination. Later, if considered necessary, a further sample was requested within 24 or 48 hr. Thereafter specimens were examined at approximately 5-day intervals until the case was discharged from hospital. All specimens were plated on McConkey, Difco 'S.S.', and Wilson Blair media, and if any non-lactose fermenters were present they were investigated in the routine manner. In searching for the two types of coliform organism a representative amount of growth was collected by platinum loop from the plate, and emulsified in a drop of diluted antiserum on a squared perspex sheet. If agglutination occurred individual colonies were next tested, and those showing positive slide agglutination were subcultured for further biochemical and serological investigation. If the composite material gave a negative agglutination test single colonies were again examined and, if agglutinated, investigated further.

The cultural characteristics of the  $\alpha$  type have already been described. The  $\beta$  variety has no distinctive feature when grown on agar or blood-agar plates, since the colonies do not differ from the normal coliform types and no characteristic odour,

like that of the  $\alpha$  organism can be detected. In sugar media most strains produce acid and gas in lactose, glucose and mannitol within 24 hr., though not infrequently a strain will produce acid first and gas later; dulcitate is not changed on the 1st day, but yields acid and gas after 7 days' incubation; salicin gives acid in the 1st day only, some strains producing acid and gas within 7 days; from maltose an occasional strain produces acid on the 1st day after incubation, but most strains show only a weak acid production even on the 7th day. In sorbitol media an occasional strain produces acid. All strains produced indole and none  $H_2S$ ; they do not liquefy gelatin; they give negative Vosges-Proskauer tests and positive methyl-red reactions; none seem to be able to utilize citrate, and although all grow at 44° C. with acid production, only a few strains produce a small amount of gas within 48 hr.  $\beta$  strains are motile when observed under suitable conditions. Motility

still capable of absorbing the 'B' agglutinins. Therefore, when the heated culture is used, the 'O' and the 'B' agglutinins can both be absorbed from a serum prepared against a formalized or living culture. As another strain with the same 'O' and a different 'B' is not available it has not been possible to prepare a pure anti-'B' serum. On the other hand, the  $\beta$  organisms when suitably cultured can develop marked motility, and antisera prepared from these forms certainly contain more 'H' agglutinins than do sera prepared from ordinary strains, and this can be demonstrated by agglutination tests carried out at 55° C. In fact, if the ordinary cultures are used to absorb a serum containing 'H' and 'O' agglutinins the 'O' are absorbed but the 'H' are not. When, however, the strains are passaged, and markedly motile forms are developed, then both 'H' and 'O' agglutinins are absorbed. Suitable emulsions of the strains used, either unheated or heated, completely

Table 1. Admissions to cubicle unit, 1948

	Gastro-enteritis			Other conditions		
	Fatal cases	Recovered cases	Doubtful cases	Recovered cases	Deaths	Total
Jan.	1	2	2	9	0	14
Feb.	1	7	1	10	1	20
Mar.	1	4	0	14	1	20
Apr.	1	5	0	13	1	20
May	3	4	1	9	1	18
June	4	9	0	6	1	20
July	2	4	1	9	0	16
Aug.	4	3	2	6	0	15
Sept.	2	2	0	13	0	17
Oct.	1	7	1	14	0	23
Nov.	0	1	0	9	0	10
Dec.	4	3	0	7	0	14
Totals	24	51	8	119	5	207

can most easily be demonstrated by passage through a Craigie tube, and is often maintained for a long period at room temperature.

For the preparation of sera for serological identification, the technique described by Kauffmann (1947) has been used. There has been no difficulty in identifying the 'O' or heat-stable fraction and no difficulty in preparing antigens and sera for the complete organisms, but identification of the 'K' antigens of Kauffmann has been difficult. Both  $\alpha$  and  $\beta$  were sent by Dr Giles to Dr Kauffmann at the State Serum Institute at Copenhagen, who has found that they do not belong to any of the serological types of *Bact. coli* described by him and the Scandinavian workers, and has suggested that the  $\alpha$  type be given the antigenic formula O 110 B4 and the  $\beta$  type O 111 B5. According to Kauffmann (1947) the 'B' antigen of the coliform organisms is thermostable but when heated is not agglutinable, though it is

remove all the agglutinins from sera prepared for the 'O' antigen.

#### EPIDEMIOLOGY

The classification of 207 cases admitted to the cubicle unit in 1948 is given in Table 1, from which it is seen that admissions were lowest in November, December and January. Seventy-five of these cases were classified definitely as gastro-enteritis, and on clinical grounds eight more might or might not have been the same disease. The prevalence is in marked contrast to the epidemic year 1947, when out of a total of 415 so admitted, 207 were diagnosed as infective gastro-enteritis. In 1948, nosocomial infantile infections were infrequent, whereas in 1947, 58.9% of cases contracted their infection in some institution. The total deaths in the 1948 series was 24 out of 75 (32%) of undoubted cases, and 24 out of 83 (27.6%) when doubtful cases are included.

There were five deaths among 124 cases admitted to the cubicle unit and not classified as suffering from gastro-enteritis. These cases comprised 41 with feeding upsets, 29 with Sonne dysentery, 28 with respiratory infection, 3 with an *S. typhi murium* infection, 3 with pyloric stenosis, and 20 with miscellaneous conditions. The five fatal cases died of pneumonia, coeliac disease, erythroblastosis, gangrene of feet and Sonne dysentery. The child who died of Sonne dysentery contracted the infection 4 days after birth, being infected by the mother who was a carrier.

THE INCIDENCE OF GASTRO-ENTERITIS  
IN RELATION TO AGE GROUPS, AND  
TYPES OF *BACT. COLI*

Details of age distribution and of types of *Bact. coli* present in the faeces of actual cases of gastro-enteritis are given in Table 2. As usual, the disease

death seemed in each case to be infantile, gastro-enteritis.

In the group of 75 clinically positive cases, in one of which no bacteriological examination was made, a total of 60 (81%), showed the presence of the  $\alpha$  or  $\beta$  types. If those cases with doubtful symptomatology are included, the percentage yielding positive cultures was 75.

The frequency of the recovery of the  $\alpha$  and  $\beta$  types of organisms from faeces in relation to days after admission is given in Table 3. Within 4 days of admission 13 cases showed the  $\alpha$  type and 19 the  $\beta$  type, 4 and 5 cases respectively did so between 5 and 7 days after admission, and 8 and 13 cases respectively at a later date. There are considerable technical difficulties in recovering the  $\alpha$  or  $\beta$  types from faecal cultures, since if the colonies of the agglutinable type are very scanty it is difficult to pick them out from amongst a large number of inagglutinable colonies. Furthermore, specimens taken

Table 2. Incidence of gastro-enteritis in relation to age groups, and types of *B. coli*

Months	$\alpha$ positive cases		$\beta$ positive cases		$\alpha$ and $\beta$ negative cases		Not examined	Doubtful cases		Total cases
	Recovered	Fatal	Recovered	Fatal	Recovered	Fatal		Recovered	Type	
0-1	0	0	1	0	0	2	0	0	0	3
1-2	3	4	1	2	1	0	0	1	0	12
2-3	6	0	4	2	1	1	1	0	0	15
3-4	3	1	7	5	3	0	0	0	0	19
4-5	3	0	3	0	0	0	0	0	0	6
5-6	0	0	2	1	1	0	0	1	0	5
6-7	2	0	0	0	1	0	0	0	0	3
7-8	1	1	2	0	0	0	0	1	0	5
8-9	0	0	1	1	0	0	0	0	0	2
9-10	1	0	2	0	0	0	0	0	0	3
10-11	0	0	0	0	1	0	0	0	0	1
11-12	0	0	0	0	0	1	0	2	1 $\beta$	3
12-24	0	0	1	0	0	2	0	3	1 $\beta$	6
Totals	19	6	24	11	8	6	1	8		83

showed a greater incidence among children between the ages of 1-5 months, and after 5 months there was a considerable decrease, though cases still occurred even up to 2 years.

The  $\alpha$  type of *Bact. coli* was recovered from 25 cases, 6 of which were fatal, while the  $\beta$  type was recovered from 35 cases, 11 of which ended fatally. In 14 cases which were clinically suffering from gastro-enteritis with six deaths, neither of these organisms was recovered. Of 8 cases which, on clinical grounds, were somewhat doubtful on account of the mildness of the disease, as might be expected in a disease of such varying severity, 2 yielded the  $\beta$  variety. In one fatal case which died on admission to hospital no bacteriological examination was made. In the age-group 9 months to 2 years there were three fatalities in children from whom neither of the *Bact. coli* types could be recovered, but the cause of

Table 3. Bacteriological examination of faeces

Isolations of  $\alpha$  and  $\beta$  types *B. coli* in relation to time after admission.

Days after admission	$\alpha$ positive cases		$\beta$ positive cases	
	Fatal	Recovered	Fatal	Recovered
1-4	3	10	5	14
5-7	3	1	1	4
8-14	0	6	2	3
15-22	0	0	1	5
23+	0	2	2	0
	6	19	11	26
Total	25		37	

during a subacute phase of the disease may often give negative results. When, however, an acute relapse occurs the organism appears in almost pure culture and there is then no difficulty in isolating it.

In certain cases this irregularity of excretion has suggested that a child might have been admitted with one type of infection, with comparatively mild symptoms, and despite isolation in a cubicle has been cross-infected, developing a more acute disease from which the  $\alpha$  or  $\beta$  type could be recovered. Of the 19  $\alpha$  positive cases which recovered only 3 were positive when discharged from hospital on the 104th, 32nd and 14th days respectively after admission, while in the  $\beta$  positive series of 26 recovered cases, 4 still showed the organism before leaving hospital on the 41st, 19th, 13th and 8th days respectively. There seems to be no doubt, therefore, that in most cases the  $\alpha$  and  $\beta$  types of *Bact. coli* disappear from the faeces as convalescence is established, although a certain proportion of cases remain carriers for variable periods. Of the 124 cases with other diseases admitted to the cubicle unit for observation, none showed the presence of either types of *Bact. coli* in their faeces. During the first half of the year there were seventeen  $\alpha$  and eight  $\beta$  positive cases, whereas during the second half there were two  $\alpha$  and eighteen  $\beta$  positive cases.

was recovered three times, and the  $\beta$  type twelve times. The  $\alpha$  type was not recovered from children over 1 year old or from adults. The  $\beta$  type was isolated three times from the 141 specimens in this category examined. Table 5 gives a summary of the particulars of the patients giving positive results, and from this it is seen that for the most part the patients gave quite suggestive histories of gastro-enteritis. Three cases actually died of this disease, 11 more had symptoms of intestinal upset, and 4 were contacts of cases but were apparently perfectly well.

## DISCUSSION

From the foregoing, and from the results recorded in previous papers, it would appear that there must be some relationship between the presence of certain types of coliform organisms in the faeces and gastro-enteritis in infants. It is not possible to state what this relationship actually means, because so far there is no certain method available of reproducing gastro-enteritis in the lower animals with the organisms isolated from human beings. There is very little

Table 4. *Results of examination of faeces specimens from 390 cases in wards and institutions not set aside for gastro-enteritis*

	Under 1 year		Total negative	Over 1 year		Total negative
	Positive $\alpha$	Positive $\beta$		Positive $\alpha$	Positive $\beta$	
City hospital: Ward 1	1	1	19	0	0	8
Ward 6	0	1	19	0	0	7
Wards 7 and 8	0	0	7	0	2	14
Ward 9	0	0	9	0	0	12
Thorngrove nursery	0	3	20	0	1	2
Middlefield nursery	0	0	2	0	0	26
Westfield nursery	0	0	0	0	0	5
Charlotte Street nursery	0	0	0	0	0	2
Maternity nursery	0	0	4	0	0	0
R.H.S.C.	1	3	19	0	0	0
Various	1	4	25	0	0	172
	3	12	124	0	3	248

#### INCIDENCE OF $\alpha$ AND $\beta$ TYPES IN CHILDREN AND ADULTS SUFFERING FROM INTESTINAL UPSET DUE TO VARIOUS ETIOLOGICAL CAUSES

During the year faeces from 390 individuals were examined to obtain further information about the distribution of these special types of coliform organisms. These specimens were obtained from children and adults in other wards of this hospital, from other hospitals and institutions, and from patients under the care of private practitioners, and were sent for examination on account of some degree of upset of the intestinal tract. The results of these examinations are shown in Table 4. From 139 specimens from children under 1 year of age the  $\alpha$  type

evidence that the presence of the  $\alpha$  or  $\beta$  types in the intestine causes any production of demonstrable antibodies, such as agglutinins. If the disease were entirely due to absorption of toxins from the intestine agglutinins might not be produced. If the work of Theobald Smith (1927) on white scour in calves indicates that coliform organisms can, under special circumstances, become pathogenic in the intestine, they might do so in infants. Infantile gastro-enteritis is a disease of the very young, and for some reason it is much more frequently associated with bottle than with breast-fed children. The faeces from a number of cases of intestinal infections such as Sonne and Flexner dysentery, typhoid and paratyphoid fever, and ulcerative colitis have been examined for the presence of the  $\alpha$  and  $\beta$  organisms,

but with negative results except in one instance in which a number of infants in a nursery were cross-infected, some yielding both *Bact. sonnei* and the  $\alpha$  type of *Bact. coli*. Other types of coliform bacilli may be found to be associated with the disease. The  $\alpha$  type of organism was almost always present in the early part of 1947, and then in the autumn of that year the  $\beta$  organism appeared in a significant number of isolations. In the present series of cases the  $\alpha$  type was fairly prevalent in the first half of the year along with the  $\beta$  organism, while in the second half of the year only 1 case infected with the  $\alpha$  type was encountered. In the present year (1949) 1 case of  $\alpha$  infection has so far been found, all the other cases showing the  $\beta$  type. Further, through the co-operation of Dr Joe, of the City Hospital, Edinburgh,

in the Report on Neonatal Mortality and Morbidity (1949).

## SUMMARY

1. The association of the  $\alpha$  type of *Bact. coli*, an organism similar to *Bact. neapolitanum* or to D433 Taylor, Powell and Wright, with gastro-enteritis of infants is further confirmed.

2. A new type of *Bact. coli* called the  $\beta$  type and differing from the  $\alpha$  type in certain of its cultural characteristics and antigenically is described as also associated with cases with similar symptoms.

3. The antigenic structure of the  $\alpha$  and  $\beta$  types has been examined, and both types show a well-marked specificity.

4. Either organism can be readily isolated from

Table 5.  $\alpha$  and  $\beta$  positive cases from series of 390 individuals with intestinal upset due to various etiological causes

Date	Place	Initials	Age	Type	Brief history
19. iii. 48	Ward 1	D. I.	7 months	$\alpha$	Diarrhoea and vomiting. Transferred to Ward 10
28. iii. 48	R.H.S.C.	B. S.	9 months	$\alpha$	Frequent loose stools
30. iii. 48	Outside	R. F.	8 months	$\alpha$	Diarrhoea and vomiting. Admitted to hospital
28. v. 48	Ward 1	R. D.	11 weeks	$\beta$	Developed diarrhoea and vomiting. Transferred. Died G.E.
10. vii. 48	Ward 6	C. R.	6 weeks	$\beta$	Whooping cough. Diarrhoea and vomiting
26. viii. 48	Wards 7 and 8	M. R.	2 years	$\beta$	Sisters—both well
26. viii. 48	Wards 7 and 8	W. R.	1 year 2 months	$\beta$	
1. ii. 48	Thorngrove nursery	I. D.	3 months	$\beta$	Diarrhoea and Vomiting. Transferred. Died G.E.
1. ii. 48	Thorngrove nursery	B. D.	3 months	$\beta$	Brother of previous case. Not ill
1. ii. 48	Thorngrove nursery	A. J.	2 months	$\beta$	Contact with above. Not ill
16. viii. 48	Thorngrove nursery	W. S.	1 year 2 months	$\beta$	Loose stools for several days. Sent home
11. ii. 48	R.H.S.C.	J. McK.	5 months	$\beta$	Loose stools and vomiting
25. ii. 48	R.H.S.C.	R. F.	7 months	$\beta$	Loose stools 3 days
12. vi. 48	R.H.S.C.	J. Z.	3 months	$\beta$	Vomiting and diarrhoea
26. ii. 48	Outside	W. B.	5 months	$\beta$	Diarrhoea and vomiting. Collapsed and died
1. ix. 48	Outside	B. D.	10 months	$\beta$	Green stools and sickness
1. ix. 48	Outside	D. G.	9 months	$\beta$	Diarrhoea and vomiting
29. ix. 48	Outside	F. D.	8 months	$\beta$	Diarrhoea and green stools

80 specimens of faeces, from children under 1 year and admitted to hospital with a diagnosis of gastro-enteritis, were examined and 22 showed the  $\alpha$  type, and none the  $\beta$  type. Three other cases were between 1 and 2 years, and 1 case between 2 and 3 years, but the specimens from them failed to show either of the types of *Bact. coli* associated with gastro-enteritis. It seems, therefore, that the incidence of the different varieties of *Bact. coli* varies in different parts of the country. The  $\alpha$  and  $\beta$  organisms are easily identified by specific slide agglutination tests, and may serve as useful organisms in studies of intestinal cross-infection, a situation which often arises from inadequacy of nurses, and which has been emphasized

faeces during an acute phase of the disease, but there may be some difficulty in recovering either during a subacute stage.

5. Both organisms usually disappear from the intestinal contents as convalescence is established, but a certain number of cases have been discharged still carrying them.

6. These organisms can rarely be found in faeces from infants ill with diseases other than infantile gastro-enteritis, or in specimens from adults. When they are found the cases from which they were isolated have usually some history of contact with gastro-enteritis.

7. The actual significance of the association of



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these organisms with gastro-enteritis has not been determined, but their isolation points to a diagnosis of infantile gastro-enteritis.

8. In cubielized and open wards the examination

of faecal specimens for these organisms provides a means of controlling nursing technique and determining whether or not intestinal cross-infection has occurred.

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(MS. received for publication 18. vi. 49.—Ed.)