THE ROLE OF ENTEROPATHOGENIC BACTERIA AND VIRUSES IN ACUTE DIARRHOEAL DISORDERS OF INFANCY AND CHILDHOOD IN JOHANNESBURG

I. SUMMER DIARRHOEA

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The summer epidemics of acute diarrhoeal disorders which affect African children in Johannesburg conform to the classical pattern which has been observed for centuries in the urban populations of temperate zones.[1]

These epidemics commence every year about 4 weeks after atmospheric temperatures have risen to summer levels; they reach a climax 8 - 10 weeks later and gradually decrease in intensity before the end of summer, irrespective of atmospheric temperatures. Nearly 80% of the affected children admitted to hospital are under 1 year of age. Malnourished bottle-fed infants suffer more severely in these epidemics than well-nourished infants. Shigella and salmonella organisms can be isolated from up to 50% of the severe cases. At the beginning of summer shigellae occur 8 - 10 times more frequently than salmonellae, but this ratio decreases as the epidemic progresses and towards autumn these organisms are recovered from the stools of patients in approximately equal numbers.

Classical summer diarrhoea was eradicated from most parts of Europe and the USA about 30 years ago. Therefore, recent reports on summer diarrhoea emanating from these countries refer mainly to sporadic cases of diarrhoea occurring during the summer period, or to localized outbreaks, usually in closed communities such as schools and hospitals. Shigellae and salmonellae are rarely responsible for such outbreaks, which have been attributed to

* Deceased.

enteropathogenic strains of *E. coli* (EEC) or enteroviruses.

Very little is known about the role played by EEC or viruses in classical summer diarrhoea, and the present investigation was intended to throw further light on this subject.

MATERIALS AND METHODS

During the period October 1959 - March 1960, rectal swabs were taken from 60 children with severe diarrhoea (group 1), from 60 unselected children with diarrhoea that occurred at the same time of year (group 2), and from 60 controls not suffering from diarrhoea (group 3). All were children with ages ranging from 1 month to 9 years, seen at Baragwanath Hospital.

Group 1 was composed entirely of patients who showed clinical evidence of dehydration and had been admitted to the emergency centre of the hospital to receive intravenous fluid therapy. Group 2 consisted of an unselected series of children who presented themselves during the summer at the outpatient department with diarrhoea as their main complaint. Most of these patients were suffering from mild attacks of diarrhoea and only about 5% could be considered to be affected as severely as the patients in group 1. The normal controls in group 3 were children not suffering from diarrhoea who were attending the surgical outpatient department for a variety of complaints.

These groups were not matched for age, which ranged from 1 month to 9 years. In group 1 there were 58 patients aged 1 year or less, and of these 19 were aged 4 months or less; in group 2 there were 51 patients aged 1 year or less and of these 9 were aged 4 months or less; and in group 3 there were 46 patients aged 1 year or less and of these 11 were aged 4 months or less.

Two rectal swabs were taken from each subject included in this series. One of these was spread on 'S.S.', desoxycholate, and MacConkey agar plates, and inoculated into selenite F enrichment medium, from which a further desoxycholate plate was spread after 24 hours' incubation.

After overnight incubation at 37°C., non-lactose-fermenting colonies were selected for biochemical and serological investigation with a view to identifying members of the salmonella or shigella groups. Lactose-fermenters on MacConkey agar were screened by slide-agglutination of at least 4 colonies per plate with polyvalent EEC antisera. Agglutinable colonies were transferred to tubes of nutrient broth, which were subsequently tested by tube-agglutination against the individual EEC antisera.

The swab taken for virus studies was agitated in 2·0 ml. of Hanks' solution containing 0·5% lactalbumen hydrolysate, with penicillin, streptomycin and neomycin. This fluid was stored at approximately 4°C. during transit to the virus laboratory, where it was frozen at —20°C. until tested. After thawing at 37°C., fluids were inoculated into primary cultures of vervet monkey kidney, which were then observed for at least 14 days. In parallel with the tissue cultures, 24-hour-old white mice were inoculated intraperitoneally and observed for 14 days.

Isolates in tissue culture were tested against standard enterovirus antisera, including ECHO virus types 1 - 28.

RESULTS

A summary of the results is given in Table I. Enteropathogenic *E. coli* (EEC) were isolated from the specimens obtained from 26 (43·3%) of the severe cases, from the same number and percentage of the unselected cases, and from 14 (23·3%) of the normal controls. The distribution of the various EEC is shown in Table II. The strain O125:B15, which is of doubtful pathogenicity, was isolated much more frequently than any of the other 8 serotypes. Excluding the strain O125:B15, EEC were found in 18 (30·0%) of the severe cases, 17 (28·3%) of the unselected cases and 7 (11·7%) of the normal controls.

Shigellae were grown from the stools of 16 (26·7%) of the severe cases, from 7 (11·7%) of the unselected cases and from 1 (1·7%) of the normal controls. Salmonellae were isolated from the stools of 6 (10%) of the patients in group 1 and from 1 patient (1·7%) in each of groups 2 and 3. No attempt was made to type the salmonellae.

Viruses were isolated from the stools of 11 (18·3%) of the

TABLE I. TOTAL ENTEROPATHOGENIC BACTERIA AND VIRUSES ISOLATED FROM PATIENTS AND CONTROLS

	Children		
	Group 1	*Group 2*	*Group 3*
Enteropathogenic *E. coli* (EEC):			
Cases positive	26 (18*)	26 (17*)	14 (7*)
Percent positive	43·3 (30·0*)	43·3 (28·3*)	23·3 (11·7*)
Shigellae:			
Cases positive	16	7	1
Percent positive	26·7	11·7	1·7
Salmonellae:			
Cases positive	6	1	1
Percent positive	10·0	1·7	1·7
Viruses:			
Cases positive	11 (12**)	16 (19**)	18
Percent positive	18·3	26·7	30·0

*EEC excluding O125:B15.
**Total virus isolates.

TABLE II. ENTEROPATHOGENIC *E. coli* STRAINS ISOLATED

	026: B6	*055: B5*	*086: B7*	*0111:B 4*	*0119: B14*	*0125: B15*	*0126: B16*	*0127: B8*	*0128: B12*	*Total*
Group 1	1	1	2	3	1	8	4	4	2	26 (18*)
Group 2	2	5	1	1	2	9	2	3	1	26 (17*)
Group 3	1	0	1	2	0	7	1	0	2	14 (7*)

*EEC excluding O125:B15.

TABLE III. VIRUSES ISOLATED INCLUDING DOUBLE ISOLATES

	Coxsackie	*Polio*	*Echo types*										*Unidentified*	*Total*
			1	*2*	*4*	*6*	*7*	*12*	*13*	*14*	*15*	*20*		
Group 1 (severe diarrhoea) ..	6	1				2	1			1			1	12
Group 2 (unselected diarrhoea) ..	6	2	5	2		1	1						2	19
Group 3 (controls)	6	1	2	1	1			1	1	2	1	1	1	18

patients suffering from severe diarrhoea, from 16 (26·7%) of the unselected patients and from 18 (30·0%) of the normal controls. A detailed list of the viruses is given in Table III. It will be noted that ECHO viruses were encountered on 4 occasions in group 1, on 9 occasions in group 2 and on 10 occasions in group 3.

An analysis of the patients whose faeces contained more than one pathogenic or potentially pathogenic organism is shown in Table IV. Multiple infection was most common in group 1 and least common in group 3. This distribution was predictable, because group 1 had the highest total number of EEC, shigellae and salmonellae, and group 3 contained the lowest number of these organisms. The chances for multiple infection occurring were thus highest in group 1.

TABLE IV. MULTIPLE INFECTIONS WITH BACTERIA AND/OR VIRUSES

	Children		
	Group 1	*Group 2*	*Group 3*
Enteropathogenic *E. coli:*			
With shigellae only ..	6 (3*)	1 (0*)	1 (0*)
With salmonellae only ..	4 (2*)	1	0
With shigellae and viruses ..	1	2 (1*)	0
With viruses only	4	8 (6*)	4 (3*)
Shigellae:			
With viruses only	1	1	0
Salmonellae:			
With viruses only	0	0	1

*EEC excluding O125:B15.

DISCUSSION

This investigation has confirmed our previous finding that many of the severe cases of summer diarrhoea are caused by shigella and salmonella organisms.[1] In both studies the incidence of these organisms was considerably lower in unselected groups of children who were suffering, for the greater part, from mild diarrhoea, and their incidence was negligible in normal controls. These findings strongly suggest that infection with shigella and salmonella organisms usually leads to severe attacks of diarrhoea in young children and that unaffected carriers are rare among them.

The role of EEC in the summer epidemic is difficult to evaluate. The strain O125:B15 outnumbered all others, but its incidence was practically the same in diarrhoeal and in normal stools. In a further investigation (Part III of this report),[2] this strain was isolated more often from normal controls than from children suffering from diarrhoea. Similar observations have been made elsewhere.[3] For these reasons, and in view of the fact that this strain has apparently never been reported as the main cause of any outbreak,[4] it seems advisable to regard it as being of negligible pathogenicity. Excluding this strain, the incidence of EEC was almost 3 times higher in patients suffering from diarrhoea than in normal controls, and this strongly suggests that they play an important part in the summer epidemics.

It should be noted that the incidence of EEC was practically the same in patients with severe attacks and in those with attacks of moderate severity, and also that these organisms were present in over 10% of normal controls. One must therefore assume either that wide variations exist in the virulence of EEC, even of the same strain, or that there are marked differences in the susceptibility of individual hosts. The high incidence of EEC in normal controls makes it difficult to define the extent to which these organisms are concerned in the summer epidemics with the same accuracy as, for example, in the case of shigellae.

Our results give no indication that viruses play a significant part in severe summer diarrhoea, either alone or in combination with enteropathogenic bacteria. Enteroviruses, particularly those of the ECHO group, have recently been reported as a cause of mild summer diarrhoea in the USA.[5] In our survey they were more common in normal controls than in patients suffering from diarrhoea. Had the reverse been the case, we would almost certainly have attributed significance to this finding. The low isolation rate of viruses in group 1, i.e. the severely affected children, can be explained on the basis of the larger number of children in this group under the age of 4 months. In these young children, colonization of the bowel with viruses may be impaired by immunity transmitted from the mothers.[6] It is noteworthy that the incidence of EEC, against which there is practically no passive transfer of immunity from the mother,[7] was the same in infants of 4 months or less and in older infants.

SUMMARY

A comparison was made between 60 children with severe diarrhoea, 60 unselected children with diarrhoea, and 60 normal controls. These comprised African children aged from 1 month to 9 years, seen at Baragwanath Hospital during the summer months from Oct. 1959 to March 1960.

Shigella and salmonella organisms were recovered from 22 (36·7%) severe cases, 8 (13·3%) unselected cases, and 2 (3·3%) control cases.

Enteroviruses were isolated from 11 (18·3%) severe cases, 16 (26·7%) unselected cases, and 18 (30·0%) control cases.

Enteropathogenic *E. coli* (excluding strain O125:B15) were found in 18 (30·0%) severe cases, 17 (28·3%) unselected cases, and 7 (11·7%) control cases.

It is concluded that shigella and salmonella organisms play a definite part in the causation of summer diarrhoea,

particularly in severe cases. The role of EEC is less easily defined, but these organisms are undoubtedly an important cause of mild and severe diarrhoea. Known enteroviruses probably have little or no significance in the aetiology of the local summer epidemics.

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THE ROLE OF ENTEROPATHOGENIC BACTERIA AND VIRUSES IN ACUTE DIARRHOEAL DISORDERS OF INFANCY AND CHILDHOOD IN JOHANNESBURG

II. 'NON-SPECIFIC' GASTRO-ENTERITIS

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There are marked seasonal variations in the incidence and severity of the diarrhoeal disorders which affect African children in Johannesburg. In a previous investigation[1] it was shown that severe cases requiring intravenous fluid therapy are about 20 times more common in summer than in winter, and that up to 50% of them may be caused by infection with shigella and salmonella organisms. The severe cases form a highly selected group, which even in midsummer accounts for only about 5% of the children coming to the outpatient department of the hospital with diarrhoea as their main complaint. In unselected patients, strains of shigellae and salmonellae have been found in less than 20% during the summer and in only about 8% during the winter.[1] The cause of the diarrhoea in the remaining patients has been obscure. The present study was undertaken to determine the role of enteroviruses, enteropathogenic *E. coli* (EEC), or combinations of these in 'non-specific' diarrhoea where infection with shigella or salmonella organisms could be excluded.

MATERIALS AND METHODS

The study was carried out during the winter of 1959 (June - August) and the summer of 1959 - 1960 (September - March), on a total of 310 unselected children suffering from diarrhoea and 221 matched controls. Children with diarrhoea were chosen at random from the outpatient waiting room, and this group therefore comprises patients with diarrhoea of all grades of severity; but not more than 5% of them were in need of intravenous fluid therapy. Children with normal stools attending the paediatric surgical outpatient department served as controls. Ages ranged from 1 month to 9 years, but the majority of children in each group were under 2 years of age.

* Deceased.

Technical details of the laboratory studies are the same as those given in Part I of this report.[2] Rectal swabs from all patients and controls were tested for shigella and salmonella organisms. Owing to factors beyond our control, EEC studies had to be confined to the summer months, and were carried out on only 102 patients with diarrhoea and 71 controls. Virus investigations were done on all children: primary tissue cultures of vervet monkey kidney were inoculated with faecal suspensions made from rectal swabs, and 24-hour-old white mice were inoculated in parallel.

RESULTS

Table I shows the recovery of shigella and salmonella organisms from all children examined. The incidence in control children was just under 2% in winter and summer, but in patients with diarrhoea it rose from 5·3% in winter to 15·3% in summer. The present study is concerned with the remaining children who were not infected with shigella or salmonella organisms.

A summary of the virus recoveries from the stools of patients with 'non-specific' gastro-enteritis and from controls is given in Table II. During the winter, viruses were isolated from 18% of 89 children affected by diarrhoea, and from 13·5% of 52 controls. During the summer, the isolation rate of viruses rose to 25·1% in 183 children with diarrhoea and 23·6% in the control group of 165 children. The seasonal increase was most pronounced in the Coxsackie A and ECHO virus groups.

The distribution of the ECHO virus types is shown in Table III. A wide variety of agents was encountered, with a slight preponderance of ECHO types 1, 2, 6 and 7 in the diarrhoeal stools during the summer. Recovery of several viruses from the same stool specimen occurred in only

TABLE I. INCIDENCE OF SHIGELLA AND SALMONELLA ORGANISMS IN THE TOTAL SERIES

Season	*Subjects*	*Shigella*	*Salmonella*	*Total*	*'Non-specific' cases*
Winter	94 with diarrhoea	4	1	5 (5·3%)	89
	53 controls	1	0	1 (1·9%)	52
Summer	216 with diarrhoea	29	4	33 (15·3%)	183
	168 controls	2	1	3 (1·8%)	165

TABLE II. SEASONAL DIFFERENCES IN VIRUS RECOVERY FROM PATIENTS WITH 'NON-SPECIFIC' DIARRHOEA AND FROM CONTROLS

Season	Subjects	Coxsackie A	Coxsackie B	Polio	ECHO	Reovirus	Adeno-virus	Unidenti-fied	Total with virus
Winter	89 with diarrhoea	4	5		3	3		1	16 (18·0%)
	52 controls	3	3		1				7 (13·5%)
Summer	183 with diarrhoea	19	1	6	19		2	3	46 (25·1%)*
	165 controls	18	2	3	13		1	2	39 (23·6%)

* Four of these patients harboured more than one virus.

TABLE III. ECHO VIRUS TYPES ISOLATED IN WINTER AND SUMMER

Season	Subjects	ECHO virus types										
		1	2	4	6	7	12	13	14	15	16	20
Winter	3 with diarrhoea	1					1				1	
	1 control	1										
Summer	19 with diarrhoea	6	3		3	4			2			1
	13 controls	2	1	1	1		1	1	3	2		1

TABLE IV. ENTEROPATHOGENIC *E. Coli* (EEC) RECOVERED FROM 'NON-SPECIFIC' DIARRHOEA CASES AND CONTROLS IN SUMMER

Subjects	Total	Positive including 0125: B15	Positive excluding 0125: B15	0111: B4	055: B5	026: B6	086: B7	0127: B8	0128: B12	0119: B14	0125: B15	0126: B16
Diarrhoea cases	102	39 (38·2%)	27 (26·5%)	1	7	2	1	5	3	5	12	3
Controls	71	15 (21·1%)	8 (11·3%)	2	0	1	2	0	2	0	7	1

4 patients, all of whom suffered from summer diarrhoea.

Table IV shows that EEC were encountered in 38·2% of 102 children with 'non-specific' summer diarrhoea, and in 21·1% of 71 controls. The most frequent strain was O125:B15, an agent of doubtful pathogenicity. The incidence of EEC exclusive of this strain was 26·5% for children with diarrhoea and 11·3% for controls, the higher recovery rate in the former group being attributable to the greater frequency of strains O55:B5, O127:B8, and O119:B14. Stools positive for EEC contained an additional viral agent in 10 (37%) of 27 patients with diarrhoea and in 4 (50%) of 8 controls.

DISCUSSION

In this series the incidence of enteroviruses was not significantly higher in the stools of children with 'non-specific' diarrhoea than in those of controls, either in summer or in winter. This strongly suggests that known enteroviruses are not a prominent cause of diarrhoea in this population. Our results thus differ from those of Ramos-Alvarez and Sabin,[3] who were able to isolate enteroviruses from a considerable proportion of their patients with summer diarrhoea. Our findings do not, however, exclude the possibility that these agents may occasionally cause diarrhoea in sporadic cases or in localized outbreaks.

Some evidence has emerged for the aetiological role of strains of EEC in summer diarrhoea. Our data do not, however, allow us to determine with any degree of accuracy the proportion of cases of diarrhoea caused by these organisms in our local population. For this purpose our methods may not have been sufficiently sensitive, and a higher incidence in patients and controls might have been obtained with the fluorescent antibody technique. Elucidation of the role of EEC in our population is further complicated by the high carrier rate in the control group, and by the doubtful significance of the strain O125:B15.

Owing to the small number of patients harbouring more than one virus, or a virus and an EEC strain, we were unable either to refute or confirm the impression gained by other workers that a combination of organisms may give rise to severe diarrhoea.

In a previous investigation undertaken at this hospital,[1] it was noted that during the winter months 80% of the children treated for diarrhoea at the outpatient department were at the same time suffering from an acute upper respiratory infection. In most instances the diarrhoea commenced a few days after the onset of the 'cold', and this was interpreted as indicating a direct relation between the two conditions, rather than a coincidental finding. In the present study, 63·8% of the patients with winter diarrhoea also had an infection of the upper respiratory tract, whereas this was encountered in only about 2% of the controls. There was no clear indication that catarrhal conditions of the upper airways were a significant factor in summer diarrhoea. The mechanism by which upper respiratory tract infection may lead to diarrhoea requires elucidation.

SUMMARY

In the African population of South Africa, diarrhoeal disorders are commonly caused by shigella and salmonella organisms. Many cases, however, cannot be attributed to infection with these bacteria, and this investigation was undertaken to elucidate the aetiological role of enteropathogenic *E. coli* (EEC) and enteroviruses in 'non-specific' diarrhoea.

From June 1959 to March 1960 rectal swabs were examined for viral and bacterial pathogens from 310 children with diarrhoea and from 221 controls. Shigella and salmonella organisms were isolated from 38 (12·3%) children with diarrhoea, and from 4 (1·8%) controls, leaving 272 patients with 'non-specific' diarrhoea and 217 controls.

Enteroviruses were recovered during the winter from 18·0% of children with diarrhoea and from 13·5% of controls. During the summer, viruses were isolated from 25·1% of children with diarrhoea and 23·6% of controls. Viruses, therefore, were not a major cause of diarrhoea during the period covered by the study.

A number of cases of 'non-specific' gastro-enteritis were attributed to infection with EEC, but for reasons discussed in this paper it was not possible to determine accurately the proportion of cases of diarrhoea caused by these organisms.

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THE ROLE OF ENTEROPATHOGENIC BACTERIA AND VIRUSES IN ACUTE DIARRHOEAL DISORDERS OF INFANCY AND CHILDHOOD IN JOHANNESBURG

III. SPORADIC DIARRHOEA IN A PREMATURE BABY UNIT

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Despite some overcrowding, the premature baby unit of Baragwanath Hospital has been relatively free from severe outbreaks of diarrhoea. This can probably be attributed to an unusual ward procedure which ensures that nearly every infant is handled solely by its own mother, thus greatly reducing the chances of cross-infection.[1]

Sporadic cases of diarrhoea are, however, quite common. Their aetiology has not been well understood, and consequently differing views have been held regarding their prevention and treatment. For this reason it was decided to investigate the premature infants for infection with shigella and salmonella organisms, which occur in up to 50% of African children suffering from severe summer diarrhoea, as well as for enteroviruses and enteropathogenic *E. coli* (EEC), which have been isolated at other premature baby centres during outbreaks of diarrhoea.

MATERIALS AND METHODS

The investigation was carried out during the summer months, January - April 1960, on 19 premature African infants suffering from diarrhoea (group 1), and 78 unaffected control infants housed in the same unit (group 2). All of these were 10 - 14 days old. This strict matching according to age was thought to be essential for the correct interpretation of results, because infants acquire their intestinal flora from their environment during the early neonatal period, and the chances of recovering potential or actual pathogens from the stools increase during this period with every day of life.[2] Since normal infants greatly outnumbered those with diarrhoea, it was not possible to investigate all infants with normal stools in the unit, and only 3 or 4 normal matched controls were studied for every case of diarrhoea.

The premature baby unit constitutes a closed environment, and it was considered possible that even unaffected inmates might acquire an intestinal flora differing markedly from that of normal African children living at home. In order to detect such possible differences, a further unmatched control series was included (group 3). This comprised 74 mature African infants and older children who were attending the paediatric surgical outpatient department at this time. Their ages ranged from 1 month to 3 years, but 49 (66·2%) were under the age of 1 year.

The collection of specimens and the laboratory methods were the same for the infants with diarrhoea and both control groups, and all 3 series were investigated concurrently. Technical procedures were the same as those described in Part I of this study.[3]

RESULTS

The findings are summarized in Table I. No shigella or salmonella strains were isolated from the infants with diarrhoea; but 1 child in control group 2 and 1 in control

* Deceased.

TABLE I. INFECTIVE AGENTS RECOVERED FROM THREE GROUPS OF CHILDREN

	Total examined	*Shigellae and salmonellae*	*Viruses*	*EEC*									*Total EEC excluding B15*
				B4	*B5*	*B6*	*B7*	*B8*	*B12*	*B15*	*B16*	*Total*	
Group 1: Premature infants with diarrhoea	19	0	0	0	0	1	0	0	2	3	4	10 (52·6%)	7 (36·8%)
Group 2: Premature infants without diarrhoea	78	1	0	2	2	3	0	1	1	22	4	35 (44·9%)	13 (16·7%)
Group 3: Normal child population	74	1	21 (28·4%)	1	0	2	2	0	1	8	1	15 (20·3%)	7 (9·5%)

group 3 harboured salmonella organisms, although neither of these children had diarrhoea.

No viruses were recovered from the specimens collected from patients and normal controls in the unit. Viruses were isolated from 21 (28·4%) of the children living at home.

Enteropathogenic *E. coli* (EEC) were obtained from the stools of 10 (52·6%) of the 19 infants suffering from diarrhoea, from 35 (44·9%) of the 78 normal controls in the same unit, and from 15 (20·3%) of the 74 children living at home. Strain O125:B15 occurred in 3 (30·0%) of the infants positive for EEC in group 1, in 22 (62·9%) of the control infants positive for EEC in group 2, and in 8 (53·3%) of the positive group-3 children who were living at home. It was therefore encountered more frequently in children not suffering from diarrhoea.

DISCUSSION

This investigation has shown that shigellae and salmonellae could not be incriminated as important causes of sporadic diarrhoea in the premature baby unit during this period. No enteroviruses were isolated from either the patients or the controls in the unit, while the incidence of viruses in the stools of children in group 3 was relatively high. The rarity of occurrence of enteroviruses in the stools of premature babies has also been noted elsewhere.[4] Immunity transmitted from the mother, or an inhibitory substance contained in milk, may be responsible for this remarkable phenomenon. In this connection it may be noted that all infants in the Baragwanath premature baby unit received only breast milk during the period covered by this investigation.

The incidence of EEC was high in the infants in this unit, being more than twice as high in premature infants of groups 1 and 2 than in older children who were living at home. The prevalence of the strain O125:B15 in infants not suffering from diarrhoea confirms that it is of doubtful pathogenicity. This also appears to be the case in other parts of the world, where this strain occurs commonly in children with normal stools.[5]

Excluding O125:B15, EEC were found in 7 (36·8%) of 19 premature infants with diarrhoea, in 13 (16·7%) of 78 normal premature infants in the same unit, and in 7 (9·5%) of 74 older children living at home. It can therefore be concluded that residence in the unit predisposed to infection with EEC, and that in a number of infants this led to diarrhoea. It is well known that intestinal infection with EEC does not always cause diarrhoea, particularly in infants receiving breast milk, and this may have favoured the occurrence of symptomless carriers. The limited immunity of these premature infants against EEC contrasts markedly with their freedom from enteroviruses. To some extent this may be explained by the poor passage across the placenta of antibodies to EEC.[6]

SUMMARY

During the summer months of 1960, a study was made at Baragwanath Hospital of 19 premature infants suffering from diarrhoea, 78 premature infants with normal stools in the same unit, and 74 older children without diarrhoea attending the outpatient department.

No enteroviruses were isolated from any of the premature infants, although they occurred in 28·4% of the children living at home.

Shigella or salmonella organisms were not recovered from premature infants with diarrhoea. Only 1 premature infant in the normal control group and 1 child in the outpatient control group were infected by salmonella organisms.

The *E. coli* strain O125:B15 was most commonly found in the premature infants without diarrhoea. Excluding this strain, EEC were recovered from 7 (36·8%) of 19 premature infants with diarrhoea, from 13 (16·7%) of 78 premature infants without diarrhoea in the same unit, and from 7 (9·5%) of 74 older children without diarrhoea who were living at home.

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