COLONY INCOMPATIBILITY AMONG STRAINS OF *ESCHERICHIA COLI* ISOLATED DURING AN OUTBREAK OF GASTROENTERITIS IN ONE WARD

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SEVERAL explanations have been proposed to account for the Dienes phenomenon that occurs between some strains of *Proteus* (Dienes, 1946). Senior (1977) suggested that proticines played a major role in the phenomenon but that not all the results could be explained in this way.

The term "colony incompatibility" was coined by Bettelheim and Carlile (1976) to include the Dienes phenomenon of *Proteus* as well as similar interactions among other motile bacteria. Recent studies of salmonellas (Bettelheim, 1978*a* and *b*) have shown that neither serotype nor bacteriocine production nor phage type correlate with incompatibility. Although colony incompatibility is still unexplained, it was decided to examine its possible ecological significance in a hospital environment.

MATERIALS AND METHODS

Subjects. Babies from one ward in an Auckland hospital were randomly selected during an outbreak of gastroenteritis for which a viral aetiology was subsequently demonstrated.

Specimens and cultural methods. Rectal swabs from babies were sent to Wellington where tests for the presence of enteropathogenic *Escherichia coli* were carried out. Swabs were spread on MacConkey agar and 10 *E. coli*-like colonies were selected. They were identified as *E. coli* by the method of Cooke, Ewins and Shooter (1969).

Serological methods. Strains of *E. coli* were O serotyped as described by Meekin, Bettelheim and Bacon (1979) and motile strains were H serotyped by the methods of Chandler and Bettelheim (1974). Non-motile strains could not be used in this study.

Incompatibility studies. All strains were passed repeatedly through Craigie-type tubes containing semisolid agar. When fully motile as determined by microscopic observation, they were transferred to Dorset egg slopes and stored at room temperature in the dark. Strains of *Salmonella* stored in this way maintain their full motility (Bettelheim, 1978*a*). In the course of this study the strains of *E. coli* similarly maintained full motility.

Each strain studied for incompatibility was tested against each other strain on semisolid nutrient agar, in which the agar concentration was 0.35%. The plates were examined after incubation for 4 days at 37°C. Degrees of incompatibility were scored as described by Bettelheim (1978*a*) (table I) and although there is a certain amount of subjectivity in the scoring, the results are reasonably reproducible. Repeated tests on the same pair of strains usually showed differences of only one score point. Nevertheless all results described here are replicates of several tests.

RESULTS AND DISCUSSION

The results of serotyping the strains and the number of strains studied are shown in table II. When possible, more than one strain representing each serotype was chosen from each baby. The incompatibility results are shown in table III. For each strain, the total of the incompatibility scores against all the other strains was used as the "incompatibility index".

It was unfortunate that some of the serotypes studied belonged to O groups that were not

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TABLE I
System of scoring incompatibility reactions

Score	Observation
0	Total or partial merging of swarms
1	"Bunching"*, no clear zone between swarms
2	"Bunching", clear zone less than 1 mm between swarms
3	"Bunching", clear zone 1–10 mm between swarms
4	"Bunching", clear zone greater than 10 mm between swarms

* The term "bunching" is used to describe the increased density of organisms near the demarcation zone between incompatible strains.

TABLE II
Serotypes of Escherichia coli isolated from babies suffering from gastroenteritis

Baby	<i>E. coli</i> serotypes found	Number of colonies		Number not <i>E. coli</i>
		found	studied for incompatibility	
A	O1.H7	9	3	0
B	Ont.H2	1	1	8
	Ont.H19	1	1	
C	Ont.H11	1	1	9
D	O23.H16	3	3	7
E	OX8.H15	6	3	4
F	Ont.H4	10	4	0
G	O2.H4	3	2	1
	O15.H18	4	1	
	Ont.H14	1	1	
	O rough.H4	1	1	
H	Ont.H10	3	2	7

Ont = not typable with 163 O sera; OX8 = a serotype that has not yet been given an international number (Ørskov, personal communication).

typable with any accepted O antisera. However, studies now in progress in New Zealand suggest that there are indigenous serogroups that have not been found in other parts of the world.

The results shown in table III were unexpected. On the basis of the studies on salmonellas it was thought that strains of the same serotype from the same source would be compatible and show similar compatibility patterns with other strains. However, this was not the case with *E. coli*. Only the four strains from patient F, which were of serotype Ont.H4, were fully compatible and behaved mainly in similar ways with the other strains. The strains from patient A were strongly incompatible with each other although they were all of serotype O1.H7; they generally behaved similarly with other strains. The serogroup O1.H7, is exceedingly common, having been isolated from a variety of sources, human and animal, in health and disease, in many parts of the world (Bettelheim, 1978c). It is also often associated with the K1 antigen, and *E. coli* carrying this antigen is commonly associated with neonatal meningitis. If strains of this serogroup are generally incompatible with other *E. coli* and even with each other, this may explain why serogroup O1.H7 is so widespread and possibly able to replace other serotypes in a given habitat.

TABLE III
Incompatibility reactions between strains of *Escherichia coli* isolated from babies suffering from gastroenteritis

Strain	Serotype	Incompatibility scores* between indicated strains										Incompatibility index†
		A1	A2	A3	B1	B2	C1	D1	D2	D3		
A1	O1.H7	0									63.5	
A2	O1.H7	2-3	2-3	2-3	3-4	3-4	3-4	2-3	2-3	2-3	63.0	
A3	O1.H7	2-3	2	2	3-4	3-4	3-4	2-3	3	3	66.5	
B1	Ont.H2	3-4	3-4	0	0	1	1-2	3	1	2	41.5	
B2	Ont.H19	3-4	3-4	3-4	1	0	1-2	2-3	1	2-3	45.5	
C1	Ont.H11	3-4	3-4	3-4	1-2	1-2	0	2-3	0-1	1	38.0	
D1	O23.H16	2-3	2-3	2-3	3	2-3	2-3	0	2-3	2-3	52.0	
D2	O23.H16	2-3	3	2-3	1	1	0-1	2-3	0	0-1	35.0	
D3	O23.H16	2-3	3	3-4	2	2-3	1	2-3	0-1	0	38.5	
E1	OX8.H15	3-4	3-4	3-4	1-2	1	1	2-3	2	1-2	39.0	
E2	OX8.H15	3	3-4	3-4	1-2	1-2	1-2	1-2	1	1-2	40.5	
E3	OX8.H15	3	3	3-4	2-3	2-3	1-2	2-3	0	2	41.0	
F1	Ont.H4	2-3	2-3	2-3	2	2-3	2	2	2-3	1-2	38.0	
F2	Ont.H4	2-3	2-3	2-3	3	2-3	1-2	2-3	2-3	43.5	43.5	
F3	Ont.H4	2	3	3	1-2	2-3	1-2	3	3	1-2	40.5	
F4	Ont.H4	2-3	2-3	3	2-3	2-3	1-2	2-3	3	1-2	43.0	
G1	O2.H4	3-4	3-4	3-4	1	1	1	2-3	1	1-2	39.5	
G2	O2.H4	3-4	3-4	3-4	1	1	1-2	3-4	1	1-2	39.0	
G3	O15.H18	3-4	3-4	3-4	1	2-3	1-2	1-2	1	1-2	39.0	
G4	Ont.H14	3-4	3	3-4	2-3	2-3	2-3	2-3	2-3	2-3	53.5	
H1	Ont.H10	4	3	4	1-2	2-3	1	3-4	1	1	42.0	
H2	Ont.H10	3-4	2-3	3-4	1	1-2	1-2	2-3	1	1	42.5	

* See table I for explanation of scores.

† Total of mean scores for the strain.

TABLE III (continued)

Strain	Serotype	Incompatibility scores* between indicated strains												Incompatibility index†	
		E1	E2	E3	F1	F2	F3	F4	G1	G2	G3	G4	H1		H2
A1	O1.H37	3-4	3	3	2-3	2-3	2	2-3	3-4	3-4	3-4	3-4	4	3-4	63.5
A2	O1.H37	3-4	3-4	3	2-3	2-3	3	2-3	3-4	3-4	3-4	3	3	2-3	63.0
A3	O1.H37	3-4	3-4	3-4	2-3	2-3	3	3	3-4	3-4	3-4	3-4	4	3-4	66.5
B1	Ont.H2	1-2	1-2	2-3	2	3	1-2	2-3	1	1	1	2-3	1-2	1	41.5
B2	Ont.H19	1	1-2	2-3	2-3	3	2-3	2-3	1	1	2-3	2-3	2-3	1-2	45.5
C1	Ont.H11	1	1-2	1-2	2	2-3	1-2	1-2	1	1-2	1-2	2-3	1	1-2	38.0
D1	O23.H16	2-3	1-2	2-3	2	1-2	3	2-3	2-3	3-4	1-2	2-3	3-4	2-3	52.0
D2	O23.H16	2	1	0	2-3	2-3	3	3	1	1	1	2-3	1	1	35.0
D3	O23.H16	1-2	1-2	2	1-2	2-3	1-2	1-2	1-2	1-2	1-2	2-3	1	1	38.5
E1	OX8.H15	0	1	1	2	2-3	1-2	1-2	1-2	1-2	1-2	2-3	1	1-2	39.0
E2	OX8.H15	1	0	1-2	2-3	2-3	2	2	1-2	1-2	1-2	2-3	1-2	1-2	40.5
E3	OX8.H15	1	1-2	0	2-3	2-3	2-3	3	1	0-1	1-2	2-3	0-1	1-2	41.0
F1	Ont.H4	2	2-3	2-3	0	0	0	0	2	1-2	1-2	2	2	2	38.0
F2	Ont.H4	2-3	2-3	2-3	0	0	0	0	2	2-3	2	2	2	3	43.5
F3	Ont.H4	1-2	2-3	2-3	0	0	0	0	2	2	2	2	2-3	2-3	40.5
F4	Ont.H4	1-2	2	3	0	0	0	0	2-3	2-3	2-3	2-3	2-3	2-3	43.0
G1	O2.H4	1-2	1-2	1	2	2	2	2-3	0	0	0-1	3	3	2	39.5
G2	O2.H4	1-2	1-2	0-1	1-2	2-3	2	2-3	0	0	1	2	0-1	3-4	39.0
G3	O15.H18	1-2	1-2	1-2	1	2	1	2-3	0-1	1	0	2-3	1	2	39.0
G4	Ont.H14	2-3	2-3	2-3	2	2	2	2-3	3	2	2-3	0	3	2	53.5
H1	Ont.H10	1	1-2	0-1	2	2	2-3	2-3	3	0-1	1	3	0	1	42.0
H2	Ont.H10	1-2	1-2	1-2	2	3	2-3	2-3	2	3-4	2	2	1	0	42.5

* See table I for explanation of scores.

† Total of mean scores for the strain.

TABLE IV

D = with diarrhoea; H = healthy.
+ = Serotype recorded, - = not recorded in world literature.
* See text for method of calculation.

Strains of the other fully typable serogroups are not as widespread in the environment and this may be reflected in their far greater compatibility with each other. On the basis of the results obtained, all the incompatibilities of the five fully identified serotypes were added and the mean per serogroup was calculated. This arbitrary figure was taken as the "mean incompatibility index". It was highest for serotype O1.H7 which is present in most of the habitats described in the world literature (Bettelheim, 1978c, and over 500 papers published since 1944). The results are shown in table IV.

Strong incompatibility reactions with other serotypes may give some *E. coli* serotypes a greater selective advantage in a given habitat. Further studies on the mechanisms of incompatibility and on a larger variety of strains may confirm these suggestions.

SUMMARY

Strains of *Escherichia coli*, isolated during an outbreak of infantile gastroenteritis in one ward, were serotyped and tested for incompatibility against each other on semisolid agar. Serotypes commonly isolated in various parts of the world from various habitats showed a greater incompatibility than more rarely isolated serotypes, suggesting that the former may have a selective advantage and be more able to displace other serotypes in a given habitat.

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