Infantile diarrhea is a ubiquitous disease which has a high mortality rate, especially when it is unrecognized or treatment is delayed. The disease occurs not only as epidemics in nurseries but also as sporadic cases in the population.

A New Serotype of Escherichia Coli Associated With Infantile Diarrhea

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A HITHERTO undescribed *Escherichia coli* serotype (O127: B8) associated with infantile diarrhea has been isolated from 121 stool cultures from 76 patients in 3 separate epidemics in Philadelphia, Pa., Cincinnati, Ohio, and Kamloops, B. C., and from cultures from sporadic cases of the disease in Mexico City. The results of bacteriological and serologic studies of these cultures and of related *E. coli* serotypes are reported.

For more than 40 years various investigators have studied *E. coli* cultures isolated from infantile gastroenteritis patients in which no recognized pathogens, such as members of the *Salmonella* or *Shigella* groups, were found. Results of earlier investigations (for references, see *14*, *17*, *1*) were inconclusive because only biochemical methods were used in attempts to differentiate between *E. coli* cultures isolated from infants with diarrhea and cultures from normal infants. By themselves, biochemical reactions proved inadequate for this purpose since, as is now known, different *E. coli* serotypes often give identical biochemical reactions. However, the extensive investigations of Kauffmann and his associates (*15*) established methods for definitive serologic typing of *E. coli* cultures and an antigenic schema in which the micro-organisms were classified.

Bray (*2*) and Bray and Beavan (*3*) apparently were the first to emphasize the association of a particular *E. coli* serotype with outbreaks of infantile diarrhea. The same type, now labeled O111: B4 (*14*), was isolated from 42 of 44 patients who had "infantile summer diarrhea." Independently, Varela and associates (*25*) in Mexico City isolated a bacterium which they named *Escherichia coli-gomez* from an infant who died of diarrhea. Later they isolated the same serotype from other patients. Varela found that the somatic antigens of *E. coli-gomez* were identical with those of *Salmonella adelaide* (O antigen 35) and he was able to employ *Salmonella* O35 antiserum in the identification of this particular *E. coli* serotype. Further studies on the antigenic relationship of *S. adelaide* and *E. coli-gomez*, as well as proof of the identity of the latter micro-organism and *E. coli* O111: B4, were given by Olarte and

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Varela (*18*). Confirmatory investigations were made by Kauffmann (*16*) and in this laboratory (unpublished data).

The second *E. coli* serotype which has assumed importance because of its association with epidemic infantile diarrhea was described by Giles and his co-workers (*7*). Kauffmann and Dupont (*14*) found that the β serotype of Giles and associates (*7*) belonged to *E. coli* O group 55 and contained a new B antigen, B5. Smith (*21*) gave detailed descriptions of the α (O111:B4) and β (O55:B5) serotypes. Since 1945, cultures of *E. coli* O111:B4 and O55:B5 have been isolated during epidemics and from sporadic cases of infantile diarrhea in nearly all parts of the world.

Pertinent data regarding the two aforementioned serotypes are summarized in table 1. Also listed in the table are additional serotypes that were described in association with outbreaks of infantile diarrhea. As might be expected, epidemics have been reported in which *E. coli* serotypes O111:B4 and O55:B5 were not found and, in the examination of the *E. coli* flora from patients in such outbreaks, other serotypes common to the cases were reported. *E. coli* serotypes, other than those listed in table 1, also have been reported from outbreaks of infantile gastroenteritis, but further studies of their role in the disease are required. Cultures of *E. coli* O124 have been isolated repeatedly from individual patients and from outbreaks of gastroenteritis and acute diarrhea in both children and adults (*11*). For additional information on the subject of *E. coli* serotypes associated with infantile diarrhea, readers are referred to the bibliographies of the papers cited above and reference 8. Persons interested in methods for isolation and preliminary identification of *E. coli* serotypes associated with infantile diarrhea are referred to Ewing and Edwards (*13*).

The cultures of the new *E. coli* serotype were received from several sources. During 1951 and 1952, 12 cultures from 3 sporadic cases in Mexico City were received from Jorge Olarte of the Hospital Infantil. These strains were studied in connection with a cooperative study of *E. coli* serotypes in sporadic cases of infantile gastroenteritis by Olarte and Ewing (unpublished data). In December 1953, 39 cultures from 9 patients in an epidemic in Philadelphia were sent by Dr. George M. Eisenberg to Dr. Edwin Neter, Buffalo, N. Y., who forwarded them to this laboratory, and 2 cultures from 2 patients were received from Dr. Merlin Cooper, Cincinnati, Ohio. During the first 4 months of 1954 Dr. Eisenberg sent 23 cultures from 17 additional patients and Dr. Cooper sent 40 cultures taken from 40 other patients during the

Table 1. Previously described Escherichia coli serotypes associated with infantile diarrhea

Antigens			Synonyms, culture numbers, and references	
O	K	H		
111	B4	2, 12, 21, or nonmotile (−).	*Bacterium coli neapolitanum.*	Bray (*2*), Bray and Beavan (*3*).
			E. coli-gomez	Varela, Aguirre, and Carille (*25*).
			B. coli α type	Giles and Sangster (*6*).
			Type D433	Taylor, Powell, and Wright (*23*).
			B. coli, B. G. T	Rogers, Koegler, and Gerrard (*20*).
55	B5	2, 6, 7, or (−)	*B. coli* β type	Giles, Sangster, and Smith (*7*); Smith (*21*).
26	B6	11 or (−)	*E. coli* O26	Biering-Sørensen, Knipschildt, and others, quoted by Ørskov (*19*).
			E. coli O26:B6	Ørskov (*19*).
			Type E893	Charter and Taylor (*4*), Taylor and Charter (*24*).
112a, 112b	B13	18	1411–50	Ewing and Kauffmann (*9*).
112a, 112c	B11	(−)	*Shigella guanabara*	De Assis (*5*).
86a	B7	8, 9, 10, 11 or (−)	Type E990	Charter and Taylor (*4*).
119	B14	6	Aberdeen 537–52	Smith (*22*).
125	B15	19	Canioni, Vincent	Charter and Taylor (*4*).
126	B16	2	E611	Charter and Taylor (*4*).

Table 2. Biochemical reactions of Escherichia coli O127:B8 and related serotypes

Serotype	H antigens	Number of cultures examined	Glucose, lactose, mannitol	Adonitol, inositol	Sucrose	Salicin	Sorbitol	Indol MR	VP citrate H_2S	Motility
O127:B8 (4932–53, etc.)	(−)	107	AG	(−)	A, Ag (A)	(−)	[1](−)	[2](+)	(−)	(−)
O86:B7 (E990, etc.)	−, 8. 9, 10, or 11	13	AG	[3](−)	V	V	[4]AG	(+)	(−)	(+/−)
O127a, 127b:B10 (2160–53, etc.).	4	9	AG	(−)	AG	AG	AG	(+)	(−)	(+)
O86a, 86b:B9 (5017–53).	Undescribed [5]	1	AG	(−)	(−)	AG	AG	(+)	(−)	(+)
O90	(−)	1	AG	[3](−)	(−)	AG	AG	(+)	(−)	(−)

NOTE: AG, acid and gas production within 24 hours. A, acid only. (A) acid production delayed 48 hours or longer. V, variable, some strains positive, some negative.
−, test negative; +, test positive.
[1] 10 cultures produced acid from sorbitol after prolonged incubation (30 days).
[2] Indol production was weak in most strains; 72 or more hours' incubation was required.
[3] 2 cultures produced acid from adonitol. The O90 culture also fermented adonitol.
[4] 1 culture failed to ferment sorbitol.
[5] This serotype possessed an H antigen that differed from any known *E. coli* H antigen.

same outbreak. In February and April 1954, five cultures were received from Dr. E. T. Bynoe of the Laboratory of Hygiene, Ottawa, Canada. Dr. Bynoe had received the cultures from Dr. C. E. Dolman, Vancouver, B. C., who had received them from Dr. F. P. Sparks, pathologist at the Royal Island Hospital, Kamloops, B. C., where they had been isolated from 5 patients in an epidemic of 17 cases of acute gastroenteritis in infants during September 1953. In this epidemic 8 babies were seriously ill and the remainder were moderately ill, but there were no fatalities. The cases in the Philadelphia and Cincinnati outbreaks were reported to be relatively severe—3 deaths occurred in the Philadelphia epidemic and 2 deaths in the Cincinnati outbreak.

We have been informed by the above-mentioned individuals that *E. coli* serotype O127: B8 predominated in the stools during the acute stage of illness and that members of the *Salmonella* and *Shigella* groups were looked for but were not present. In some instances, serotype O127: B8 was the only coliform bacterium in the stool cultures. Cultures of this serotype were not recovered from well babies during any of the outbreaks and, to date, none has been received in this laboratory from sources other than diarrheal patients.

We understand that further data on the clinical aspects of infantile diarrhea, the extent and nature of the outbreaks, and other details concerning the epidemics in Philadelphia and Cincinnati will be published by Eisenberg and Cooper in separate reports. The details of the epidemic in British Columbia probably will be published by the investigators of the outbreak.

The other two *E. coli* serotypes described for the first time in this paper are O86a, 86b: B9, and O127a, 127b: B10: H4. Only one strain (5017–53) of O86a, 86b: B9 was available for study. This was isolated from a normal individual who was a member of the staff of a Philadelphia hospital. Nine cultures (2160–53 and so on) of the other serotype (O127a, 127b: B10) were recovered during a survey from stools of pediatric patients who did not have diarrheal disease.

Included in these studies were several strains of the previously described serotype O86: B7, which had been isolated recently from the stools of infants who had diarrhea. The cultures were forwarded to this laboratory for typing. The type strain for this serotype was received in 1950 from Dr. Joan Taylor (*4, 24*).

Biochemical Reactions

The biochemical reactions of cultures of *E. coli* serotypes O127: B8, O86: B7, and related

Table 3. The relationship of the O antigens of Escherichia coli serotypes O127:B8, O86:B7 and O group 90

O antigen suspensions (100° C., 1 hour)	*E. coli* O antiserums								
	86			90			127		
	Unabsorbed	Absorbed by—		Unabsorbed	Absorbed by—		Unabsorbed	Absorbed by—	
		127	90		127	86		86	90
86 (E990)	[1] 5, 120	1, 280	320	640	160	0	0	0	0
90	2, 560	640	0	5, 120	640	1, 280	1, 280	640	0
127 (4932–53)	1, 280	0	0	640	0	640	20, 480	20, 480	1, 280

[1] Agglutination titers are expressed as the reciprocal of the highest dilution that gave strong agglutination.

cultures are listed in table 2. The reactions of the 121 strains of serotype O127:B8—which were remarkably uniform when the geographic locations of the sources were considered—were as follows: Glucose, lactose, and mannitol were fermented with gas production within 24 hours' incubation by all cultures. All cultures fermented sucrose, but a few strains required 48 hours' incubation for the reaction to appear, and some strains did not produce gas from this substrate. Salicin, adonitol, and inositol were not attacked during 30 days' incubation. The majority of strains did not ferment sorbitol within 30 days, but 10 cultures produced acid from this carbohydrate after 30 days' incubation. Hydrogen sulfide was not detected in triple sugar iron agar, acetylmethylcarbinol was not produced, and urea was not hydrolyzed. All strains failed to grow on Simmons' citrate agar, and all were nonmotile. The methyl red test was positive. All cultures produced indole but required 72 hours' incubation or longer to produce detectable amounts of indole from 2 percent Bacto peptone water (Kovac's reagent).

Serologic Studies

The methods used for serologic studies and for antiserum production were similar to those previously reported (*9*, *10*, *12*) and were based upon methods advocated by Kauffmann (*15*).

Relationships of the O Antigens

Heated broth antigens of culture 4932–53 and other strains of the new serotype referred to as O127:B8 were tested in antiserums for all of the 126 known *E. coli* O antigen groups in dilution of 1:100. Positive agglutination reactions were obtained in antiserums for O groups 86 and 90; all other tests were negative. Upon titration of O antigen suspensions in these two O antiserums there was some variation in the titers to which the strains were agglutinated. The

Table 4. The interrelationship of the O antigens of Escherichia coli cultures of O groups 86, 90, and 127

O antigen suspensions (100° C., 1 hour)	*E. coli* O antiserums (unabsorbed)				
	86a (E990)	90	127a (4932–53)	127a, 127b (2160–53)	86a, 86b (5017–53)
86a (E990)	5, 120	640	0	5, 120	5, 120
86a, 86b (5017–53)	20, 480	640	0	10, 240	20, 480
90	2, 560	5, 120	1, 280	5, 120	160
127a (4932–53)	1, 280	640	20, 480	5, 120	0
127a, 127b (2160–53)	10, 240	1, 280	10, 240	20, 480	640

average titer was 1:1,280 in O86 antiserum and 1:640 in O90 antiserum (tables 3 and 4). When heated broth antigens prepared with cultures of *E. coli* O groups 86 and 90 were tested in O antiserums prepared with culture 4932–53 and identical strains, O group 86 cultures failed to react, whereas the antigens for O group 90 were agglutinated in dilutions of 1:640 to 1:1,280 (tables 3 and 4). The results of reciprocal agglutinin absorption tests with these O antiserums (table 3) indicated that the O antigens of serotype O127:B8 cultures were related unilaterally to those of O group 86; whereas the relationship to O group 90 was bilateral or reciprocal. However, since the O antigens of serotype O127:B8 strains were not identical with those of *E. coli* O group 90, it was decided to assign these cultures to a new *E. coli* O antigen group, namely, O127. This decision was made after consultation with Kauffmann and Ørskov of the International Salmonella and Escherichia Center, Copenhagen, Denmark.

During the study of the O antigens of cultures of *E. coli* O127:B8, a number of other cultures related to *E. coli* O groups 86 and 90 were reinvestigated. The results of agglutination tests in unabsorbed O antiserums (table 4) indicated the interrelationship of this group of cultures. Reciprocal agglutinin absorption tests indicated that the O antigens of cultures 2160–53, 2210–53, and others like these, were identical. Similar absorption tests (table 5) also showed that the O antigens of O group 127 strains and culture 2160–53 were closely related but not identical. The O antigens of these two serotypes may be expressed by the use of arbitrary formulas, as follows:

4932–53 ---------- O127a
2160–53 ---------- O127a, 127b

It should be mentioned that all of the 121 cultures associated with sporadic cases and with epidemics of infantile diarrhea belonged to O group 127a, whereas the 9 cultures like 2160–53 were O group 127a, 127b. The former group of strains were nonmotile and somewhat less active as regards their biochemical reactions, and were further characterized by a distinct B antigen, as will be shown. The nine cultures of the latter group were motile (H antigen 4).

Table 5. Relationship of the O antigens of Escherichia coli serotypes O127a:B8 and O127a, 127b:B10

O antigen suspensions (100° C., 1 hour)	O antiserums			
	127a (4932–53)		127a, 127b (2160–53)	
	Unabsorbed	Absorbed by 127a, 127b	Unabsorbed	Absorbed by 127a
127a (4932–53)	20, 480	0	5, 120	0
127a, 127b (2160–53)	20, 480	0	20, 480	10, 240

Table 6. O antigenic relationship of Escherichia coli serotypes O86a:B7 and O86a, 86b:B9

O antigen suspensions (100° C., 1 hour)	O antiserums			
	86a		86a, 86b	
	Unabsorbed	Absorbed by 86a, 86b	Unabsorbed	Absorbed by 86a
86a (2805–52)	5, 120	0	5, 120	0
86a, 86b (5017–53)	20, 480	0	20, 480	5, 120

The relationships of the O antigens of *E. coli* O86a and O86a, 86b (5017–53) are given in table 6. The relationship of these two serotypes to each other was analogous to that noted between serotypes of O group 127, mentioned above. The H antigen of culture 5017–53 was not agglutinated by H antiserums prepared with the 33 known *E. coli* H antigens. Thus, the H antigen of this serotype represented a new, unnumbered *E. coli* H antigen.

The results of reciprocal agglutinin absorption tests indicated that the O antigens of *E. coli* serotypes O86a, 86b, and O127a, 127b were related but not identical. A strong specific factor remained in each antiserum for the homologous serotype following reciprocal absorptions.

B Antigenic Relationships

When cultures of the *E. coli* serotype referred to as O127:B8 first were received in the laboratory for identification, they were tested on slides

Table 7. The relationship of the K antigens of certain Escherichia coli serotypes

K antigen suspensions	*E. coli* K (B) antiserums				
	B2	B7	B8	B10	B9
B2 (O8)	320	0	0	80	0
B7 (O86)	40	320	0	40	40
B8 (O127)	40	0	320	80	0
B10 (O127a, 127b)	0	0	0	640	0
B9 (O86a, 086b)	0	0	0	0	320
K–O90 [1]	0	40	0	80	0

[1] The K antigen of the *E. coli* O90 strain was undetermined.

with O and OB antiserum for the *E. coli* serotypes that have been associated with cases of epidemic infantile diarrhea. Living suspensions of these cultures were not agglutinated by any antiserum except that for O86, in which most of the living suspensions reacted to a greater or lesser extent. Living suspensions did not react in B7 (O86: B7) antiserum. The reason for this apparent discrepancy is believed to lie in the fact that the living O127: B8 strains were not entirely O inagglutinable and therefore reacted in the higher titered O86 antiserum and failed to react in the O86: B7 antiserum which had a relatively lower O titer. Heated broth antigens of several O127: B8 strains later were tested in O86: B7 antiserum and it was found that they reacted in dilutions of 1: 160.

Living suspensions of the new *E. coli* serotype then were tested on slides in antiserums prepared with all of the 61 known *E. coli* K antigens. Positive agglutination tests were obtained only in antiserum for B antigen 2 (O8: B2). In subsequent slide tests it was found that living suspensions of all 121 strains of serotype 127: B8 reacted in B2 antiserum. When K antigens prepared from representative strains of this serotype were titrated in serial dilutions of B2 antiserums, all strains tested reacted in this antiserum at 1: 40 but not in higher dilutions (table 7). A K antigen suspension made with a culture of *E. coli* O8: B2 did not react in OB antiserum prepared with serotype O127: B8.

The results of reciprocal agglutinin absorption tests (table 8), using living suspensions, confirmed the individuality and specificity of thermolabile somatic antigens B2 and B8. That the thermolabile somatic antigen of *E. coli* O127: B8 was in fact a B antigen was shown by absorption tests in which all agglutinins were removed from OB antiserum for O127: B8 when the antiserum was treated with a heated (100° C., 1 hour) suspension of the homologous culture (table 8). The antibody binding power of the thermolabile somatic antigen was not destroyed by heat at 100° C. Since this B antigen was not identical with, or significantly related to, any described *E. coli* K antigen, it was designated "B8."

Two other undescribed B antigens were characterized during the examination of cultures related to *E. coli* O groups 86, 90, and 127. One of these was found in culture 5017–53 (O86a, 86b) and the other occurred in the nine cultures that belonged to O group 127a, 127b. The B antigens of these cultures were not related significantly to any known *E. coli* K antigen or to those described herein (table 7). The designation B9 was assigned to the thermolabile somatic antigen of culture 5017–53 and the comparable antigen of culture 2160–53 was designated B10. That these two thermolabile somatic antigens, B9 and B10, actually were B antigens was demonstrated by appropriate absorption tests.

It was possible to prepare pure B7 antiserum by absorption of O86a: B7 antiserum with unheated or with heated suspensions of culture 5017–53 (O86a, 86b: B9). Similarly, a pure B8 antiserum was prepared by absorption of O127a: B8 antiserum with either heated or unheated suspensions of serotype O127a, 127b: B10. These absorbed antiserums agglutinated living cultures of the respective serotypes but did not react with heated suspension.

Summary

The biochemical and serologic reactions of 121 cultures of the new *Escherichia coli* serotype O127: B8 associated with infantile diarrhea are described. The cultures of the new serotype were isolated from cases of infantile diarrhea in three separate epidemics in Philadelphia, Pa., Cincinnati, Ohio, and Kamloops, B. C., and from sporadic cases of this disease in Mexico City.

Table 8. Comparison of the B antigens of Escherichia coil serotypes O127a:B8 and O8:B2

Antigen suspensions	B antiserums				
	B2		B8		
	Unabsorbed	Absorbed by O127:B8 (unheated, formalinized)	Unabsorbed	Absorbed by—	
				O8:B2 (unheated, formalinized)	O127:B8 (100° C., 1 hour)
E. coli O8:B2, unheated	320	320	0	0	----------
100° C., 1 hour	2, 560	2, 560	0	0	----------
E. coli O127a:B8, unheated	40	0	160	160	0
100° C., 1 hour	0	0	2, 560	----------	0

The O antigens of the new serotype constitute a new *E. coli* O antigen group, 127, and the thermolabile somatic antigen of the cultures was found to be an undescribed B antigen which was designated B8. All of the 121 strains of serotype O127: B8 were nonmotile.

Two other *E. coli* serotypes, O86a, 86b: B9 and O127a, 127b: B10: H4, also are described. These two serotypes were isolated from normal individuals.

The O, B, and H antigens of the new *E. coli* serotypes are compared with those of previously described *E. coli* antigens and details of the relationships noted are presented.

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Since this paper was written, and up to October 1, 1954, we have received 29 additional cultures of E. coli O127: B8, representing 43 additional cases of infantile diarrhea from outbreaks in Albany, N. Y., New Jersey, and sporadic cases in California and Chicago, Ill.

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technical publications

Distribution of Health Services in the Structure of State Government, 1950

Public Health Service Publication No. 184, parts 1–4. 1954. By Joseph W. Mountin, Aaron W. Christensen, Evelyn Flook, Edward E. Minty, Rubye F. Mullins, and Georgie B. Druzina. 360 pages; tables. $1.90.

Distribution of Health Services in the Structure of State Government, a revision of Public Health Bulletin No. 184, third edition, 1940, is now available in a single volume. The four parts—Part 1. Administrative Provisions for State Health Services; Part 2. General Services and Construction of Facilities for State Health Programs; Part 3. Personal Health Services Provided by State Government; Part 4. Environmental Health and Safety Services Provided by State Government—each released under separate cover as the data were prepared, have been bound for the convenience of the reader in a single publication, with a table of contents added. No revisions have been made in the material as previously presented in the separate parts.

State Tuberculosis Control Programs As Planned for Fiscal Years 1954 and 1955

Public Health Service Publication No. 396. 1954. 24 pages.

State program plans for tuberculosis control, fiscal years 1954 and 1955, are presented in abstract form in this booklet. Submission to the Public Health Service of a plan of operations for carrying out public health programs is required of all State agencies participating in Federal grants-in-aid for health work.

The abstracts reflect in concise form the proposed elements as described by the responsible State officials. No attempt was made to evaluate program content, and clarification of descriptions, interpretations, and editorial changes were kept to a minimum.

The booklet also presents a summary of the needs and problems significant to the tuberculosis control program as expressed by State program directors.

In addition, selected administrative information related to each State's tuberculosis control program is shown in tabular form. These data include placement of responsibility for tuberculosis control in the health department and staff assigned to the program.

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