

## ESCHERICHIA COLI O.128 CAUSING GASTRO-ENTERITIS OF INFANTS

BY

JOAN TAYLOR AND RUTH E. CHARTER

From the Salmonella Reference Laboratory, Central Public Health Laboratory, Colindale, London

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A number of cases of infantile diarrhoea had occurred in the Coventry area over a period of a few months in 1953, and in July a severely ill patient, baby Cigleris, from whom no salmonella or shigella could be isolated, was admitted to hospital. The faeces were cultured on MacConkey agar, and the primary plate sent for the serological identification of *Escherichia coli*. On examination, the plate showed a pure growth of *E. coli* of a single colonial type which was not agglutinated by any of the diagnostic sera in use at that time. Antisera were prepared with suspensions of this strain. Our investigations, which will be described in detail, showed that the somatic antigen did not belong to those already described, *Escherichia coli* O.1-O.126 (Vahlne, 1945; Kauffmann, 1954). There was no relationship to the new type O.127 investigated by Dr. W. H. Ewing (personal communication). A strain was sent to Dr. F. Ørskov at Copenhagen, who confirmed our finding, and assigned the symbols O.128:B.12 to the somatic and surface antigens.

### Source of Material

A few weeks after the Coventry strain of *E. coli* had been received, an outbreak of infantile diarrhoea occurred in the Birmingham Children's Hospital. The strains received were found to be serologically identical with the Coventry type. Altogether 86 strains were identified. Of these, 49 were isolated from infants with gastro-enteritis; 21 strains were isolated from babies whose clinical history is unknown, but who were investigated in relation to institutional outbreaks of infantile diarrhoea; one strain was isolated from the ear swab of a baby who was in a ward where an outbreak was in progress; 12 were isolated from healthy babies, of whom the majority were contacts of clinical cases, but some were from routine admission swabs; one strain came from a case of pertussis with no intestinal symptoms, and two came from healthy adults, contacts of sick babies.

Thirteen of the babies with gastro-enteritis were sporadic infections; three of these died. The areas from which strains were received were Coventry, Birmingham, Guildford, Epping, Hertford, Aberdeen, Reading, Southampton, Sheffield, Worthing, Taunton, and Greater London. It is known that outbreaks occurred in institutions in most of these towns.

The age distribution of patients with gastro-enteritis is shown in Fig. 1. Some of these cases occurred after the 49 referred to earlier, as details of age were not always available and we wished to include as many cases as possible. The maximum incidence was in babies under 1 year of age, with the bulk of cases in the 3-6 months' group. Two cases were in older children aged 2 years and 2½ years. The distribution is similar to those found in gastro-enteritis due to other serotypes of *E. coli*.

While this work was in progress, seven of the strains originally believed to be *E. coli* O.128 later were found to be closely related antigenically, but were not identical with O.128. One was isolated from a baby with measles and *Salmonella*

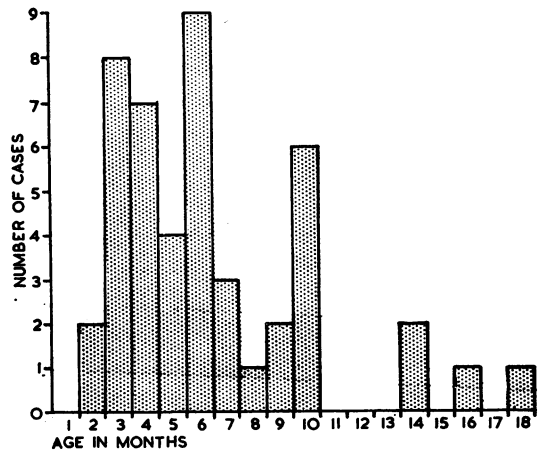


FIG. 1.—Age distribution of 46 babies with gastro-enteritis (O.128).

*typhimurium* infection; one from a baby with bronchitis but with no intestinal symptoms; four from babies with gastro-enteritis; and one from a healthy contact. Four of the last five strains were from a single nursery outbreak.

**Bacteriology**

On MacConkey agar incubated at 37° C. all 86 strains produced typical round, smooth, shiny red colonies with an entire edge. Similarly, on blood agar, the organisms showed normal smooth colonial morphology without haemolys's. On keeping the MacConkey plates on the bench it was noticed that the colonies of some strains of *E. coli* O.128 produced an intensely mucoid growth, so much mucoid material being produced that it fell on to the lid when the plate was stored in the inverted position. The Coventry strain Cigleris has been accepted as the type strain O.128, and is designated as strain No. 5594 throughout this communication. The Coventry strain, which produced a mucoid growth, was cultured on a nutrient agar to which different carbohydrates had been added to test their effect on the ability to produce a mucoid growth. The carbohydrates at a concentration of 5% were incorporated in beef extract agar. The plates were thick and dried carefully, so that surface moisture only was removed. All plates were inoculated from a four-hour beef extract broth culture of the Coventry strain. Plates were incubated at 37° C. and 22° C., and examined for mucus production after one, two, and four days' incubation. The results are shown in Table I. Mucoid material was produced more rapidly and in greater amount when cultures were incubated at 22° C. In many instances the mucoid material was first seen at the edge of a well-isolated colony, or at the edge of the confluent growth; later other

parts of the culture showed the same change, which was always maximal at the edge of the culture. Although some carbohydrates hastened the speed with which mucus was produced, and, to a certain extent increased its amount, their presence was not essential. An interesting observation was that the ability of the organisms to ferment a particular carbohydrate had no effect on the value of the carbohydrate to assist mucus production. The Coventry strain (Table I) fermented both sucrose and salicin, yet mucus was not produced on the latter medium. In order to check that no change had occurred in the organism, a sucrose agar plate was seeded from growth on a salicin plate, and vice versa. Again mucoid growth occurred on sucrose media but not on salicin. Sixty-three strains were cultured on 5% sucrose agar. After 24 hours' incubation at 37° C. five showed a mucoid change; incubation for a further two days did not increase this number. The cultures were then kept on the bench, and examined after a further 20 days. At the end of this time, four strains of *E. coli* O.128 and two of the serologically related strains still showed no production of mucoid material, six gave a doubtful appearance, and the remaining 51 were positive. Many cultures showed but few mucoid colonies, even though many of the non-mucoid colonies were well isolated. There is reason to believe that this ability to produce the mucoid material is not a property of every culture nor of each colony of a single strain.

**Microscopic Appearance**

Preparations in Indian ink were made from growth on solid media and viewed with the phase-contrast microscope. Films made from non-mucoid growth showed non-capsulated rod-shaped organisms. Material from the mucoid culture showed that practically all organisms possessed a capsule, although it was usually possible to see an occasional non-capsulated organism. A slimy matrix was visible around the capsulated organisms, which increased in amount, reaching a maximum after about five days' incubation at 22° C. When material was taken from growth which would become mucoid on further incubation, an occasional organism showed a distinct capsule, but no slime matrix was present at this stage.

Eighty-one of the 86 strains of *E. coli* O.128 were motile when grown in soft agar and incubated at 37° C.

**Serology**

The serology of this group was found to be very complicated, as four antigens required detailed study; the somatic antigen, the heat-labile surface

TABLE I  
MUCUS PRODUCTION BY *E. COLI* O.128  
(COVENTRY STRAIN)

	37° C.			22° C.		
	1st day	2nd day	4th day	1st day	2nd day	4th day
Lactose ..	-	-	-	+++	+++	+++
Glucose ..	-	-	-	++	+++	+++
Maltose ..	+	+	++	++	+++	+++
Mannitol ..	-	-	-	++	+++	+++
Sucrose ..	sl. +	sl. +	++++	++	+++	+++
Salicin ..	-	-	-	-	-	-
Dulcitol ..	+++	+++	+++	+++	+++	+++
Inositol ..	+++	+++	+++	+++	+++	+++
Sorbitol ..	+++	+++	+++	+++	+++	+++
Adonitol ..	++	++	++	+++	+++	+++
Rhamnose ..	-	-	-	++	++	++
Trehalose ..	-	-	-	+++	+++	+++
Starch ..	? +	? +	-	+	+++	+++
Nutrient agar ..	+	+	+	++	+++	+++

antigen, the capsular and slime antigen, and the flagellar antigen. Eight representative strains were chosen for detailed study:

5301	.. .. .	isolated from a sick baby in London
5594	(Coventry strain)	.. .. . Coventry
5902	.. .. .	.. .. . Birmingham
5908	.. .. .	.. .. . Guildford
5920	.. .. .	.. .. . Aberdeen
6146	.. .. .	.. .. . baby with pertussis in London
5968	isolated from a baby with measles and	<i>S. typhimurium</i> infection
6080	.. .. .	.. .. . bronchitis

The first six were shown to have identical somatic and surface antigens, whereas the last two were antigenically related to but not identical with the first six.

A number of rabbit antisera were prepared using as inoculum a living suspension of a non-mucoid strain, formalized broth cultures of motile strains, and living suspensions of a mucoid strain for the production of capsular agglutinins.

**Somatic and Surface Antigens**

The serum prepared from a living suspension was tested, using *Escherichia coli* O.1 to O.126 suspensions, and Dr. W. H. Ewing tested with O.127. No *E. coli* "O" groups were agglutinated except *E. coli* O.48, which was agglutinated to 1% of the homologous titre, and *E. coli* O.87, which showed a trace reaction. Absorption of the serum with

TABLE II  
SEROLOGICAL RESULTS ON THE SURFACE AND SOMATIC ANTIGENS

Sera	Suspension					
	Heated	Living	Heated	Living	Heated	Heated
5594	5301	5301				
	5594	5594				
	5902	5902			<i>E. coli</i>	<i>E. coli</i>
	5908	5908	5968	5968	O.48	O.87
	5920	5920				
	6146	6146				
Unabsorbed ..	6400	800	6400	400	50	trace 50
Absorbed 5902	< 50	< 25	100	< 25		
" 5301	< 50	< 25	100	< 25		
" 5968	1600	100	< 50	< 25		
" O.48	3200	800	3200	400	< 50	
" O.87	6400	800	6400	400		< 50

*E. coli* O.48 or with *E. coli* O.87 failed to have a significant effect on the homologous titre. Obviously, the serological relationship to these "O" groups is not close (Table II).

From the agglutination titres and absorption results shown in Table II, one can conclude that strains 5301, 5594, 5902, 5908, 5920, and 6146 are of the same serotype. This was confirmed, as strain 5594 removed all agglutinins from a serum made with strain 5902 and also from a serum made with 5301. It will be seen that strain 5968 was related to serotype 5594 in that the heated suspen-

sion was agglutinated to titre, but the living suspension was only agglutinated to 400 (titre 800). Absorption of serum 5594 with 5968 suspension reduced the homologous titre from 800 to 100 and from 6,400 to 1,600, using living and heated suspensions respectively. These results confirmed the conclusion that the somatic and surface antigens of 5968 were not identical with 5594, though closely related. A second strain, 6080, gave reactions identical with 5968.

The remaining 78 cultures were investigated by direct agglutination. Both heated and living suspensions were agglutinated to titre by serum 5594, but six strains were identical with 5968.

The agglutinability of the surface antigen of a living suspension was destroyed by heating to 100° C. for 30 minutes. A suspension of living organisms in normal saline so treated absorbed all somatic and surface agglutinins from the homologous serum. These tests are regarded as sufficient to class the surface antigen as belonging to the B type (Kauffmann, 1954).

**"H" Antigens**

Two "H" sera were used, the one made from strain 5902 and the second made from a strain 6 S, which is *E. coli* H.2. From Table III it is clear that strains 5594, 5902, 5301, and classical strains Bi.7455 (Vahlne, 1945) and 6 S had identical "H" structures. In addition, reciprocal absorption tests using strains and sera of 5301 and 5594 showed that these two had identical flagellar antigens.

TABLE III  
SEROLOGICAL RESULTS ON H.2\*ANTIGENS

Sera	Antigens				
	5902H	5594H	5301H	6SH	Bi. 7455H
5902					
Unabsorbed	12800	12800	12800	12800	12800
Absorbed by 5594H	< 100	< 100	< 100	< 100	< 100
" " 6 S	< 100	< 100	< 100	< 100	< 100
" " 5301H	< 100	< 100	< 100	< 100	< 100
6 S					
Unabsorbed	25600	25600	25600	25600	25600
Absorbed by Bi.7455H	< 100	< 100	< 100	< 100	< 100
" " 5902	< 100	< 100	< 100	< 100	< 100
" " 5301H	< 100	< 100	< 100	< 100	< 100

Seventy of the 86 strains were identified as having antigen H.2. Three strains, 6146 and two others, though actively motile, were not agglutinated by H.2 serum, but were found to have H.8. The two related strains 5968 and 6080 and four similar strains were motile, having *E. coli* H.10, and one strain had H.9 and one H.12. Five strains were non-motile.

**Capsule Antigen**

The relationship of capsule formation to antigenic structure was investigated. Two sera were used, one made by injecting a rabbit with the living non-mucoid strain 5301, and the second by injecting the living mucoid strain 5594 (serum referred to as 5594c).

When cultures were grown at 37° C. overnight on solid media, typical non-mucoid colonies were produced by all strains including 5594. When tested by slide and tube agglutination, using serum made with the non-mucoid strain 5301, the results were those expected—coarse rapid agglutination on slide, and in tube agglutination typical of the somatic, surface, or flagellar type, according to the suspension used.

Six strains, of which 5594 was one, when grown at 22° C. rapidly produced a mucoid growth which did not show a typical slide agglutination using serum made with non-mucoid strain 5301; the agglutination was finely granular and occurred rather slowly. This type of agglutination was shown by both the unwashed and washed suspensions. A saline suspension of the mucoid strains, when tested in tube with serum 5301, showed a general turbidity of unagglutinated cells with a deposit of a very few small agglutinated particles. The appearance was entirely different from that seen using serum 5301 and a living suspension of the non-mucoid strain or of 5594 and similar strains grown at 37° C.; here the agglutinated particles were extremely coarse and the supernatant fluid clear. It is believed that both in the slide and the tube test these agglutinated particles seen with the mucoid strains grown at 22° C. were due to the few non-capsulated organisms (as referred to previously). The capsule formation and mucus production shown by some strains grown at 22° C. have already been described.

Repeating the reactions using the serum made with the living mucoid strain 5594, it was found that suspensions of both the mucoid 5594 and non-mucoid 5301 growth were agglutinated coarsely both on slide and in tube. This serum was absorbed with a living suspension of a non-mucoid strain 5301. The results showed that agglutinins remained which agglutinated only the capsulated mucoid strain 5594 and similar mucoid strains.

Washings from non-mucoid 5301 and mucoid 5594 strains were layered on undiluted serum 5594c, and a positive ring test obtained with both. The washings were then layered on undiluted serum 5301; a positive ring test was obtained with the homologous extract but was negative with 5594

extract. Then 5594c serum was absorbed with a living suspension of 5301 and saline washing from the two strains layered on this absorbed serum; a positive precipitin reaction was given by 5594 extract, but a negative reaction by 5301 extract. Results similar to those of 5594 were given by extracts of the five mucoid strains, the extracts of

TABLE IV  
REACTIONS OF SURFACE AND SOMATIC ANTIGENS

Antigen	Sera		
	5301	5594c	5594c absorbed with 5301
5301 { slide agglutination ..	+	+	-
capsular swelling ..	-	-	-
precipitin test ..	+	+	-
5594c { slide agglutination ..	-	+	+
capsular swelling ..	-	+	+
precipitin test ..	-	+	+

All sera were used at a dilution of 1 : 5.

the non-mucoid strains behaving like those of 5301 (Table IV). The ring test was used with undiluted sera, but with sera diluted 1 : 5 it was found that the capillary tube method gave more satisfactory results.

When suspensions of the non-mucoid strain 5301 in Indian ink were mixed with the homologous antiserum and inspected with the phase-contrast microscope, no change in the appearance of the organism was observed, though agglutination occurred; similar results were obtained using the same suspension and serum 5594c. With the same technique but with a suspension of the washed capsulated strain 5594 and homologous serum, there was obvious capsular swelling and agglutination of the capsulated organisms. Serum 5594c was absorbed with a suspension of the non-mucoid strain 5301. The absorbed serum caused swelling of the capsule of strain 5594, and all other capsulated strains of the O.128 type, the only type investigated. The absorbed serum caused neither agglutination nor swelling of non-capsulated strains 5301 and others of O.128 type when viewed by phase-contrast microscopy.

From these results it is clear that the capsular antigen differs from the somatic and surface antigens already described.

The thermolability of the capsular antigen in the mucoid variant was studied. Saline suspensions of the growth from solid media of the mucoid-variant and the non-mucoid cultures were each washed twice in saline and the deposit from each centrifugalization resuspended in fresh

saline to the original volume. Each suspension was then divided into two parts and one part of each heated in a water-bath at 100° C. for 30 minutes, cooled, centrifuged, and the deposit resuspended in fresh saline.

The above suspensions were then tested for agglutinability against sera prepared from living suspensions of a normal culture of O.128 and a mucoid variant. A third serum was prepared by

TABLE V  
EFFECT OF HEAT ON THE AGGLUTINATION OF CAPSULATED STRAIN

Serum	Antigen			
	Washed Suspensions		Heated Washed Suspensions	
	5301	5594	5301	5594
5301 .. .. .	400	< 4	1600	1600
5594c .. .. .	400	200	3200	1600
5594c absorbed with 5301 ..	< 25	100	< 25	< 25

5301 = non-mucoid, non-capsulated strain.  
5594 = mucoid, capsulated strain.

absorbing the anti-mucoid serum with a non-mucoid suspension. Table V shows that the heating destroyed the agglutinability of the capsular antigen and also rendered the suspension "O" agglutinable. Similar results were obtained with other mucoid strains of *E. coli* O.128.

#### Slide Agglutination : Cross Reaction

When doing slide agglutination for the preliminary identification of cultures of *Escherichia coli* O.128, it has been found that organisms later shown to belong to this "O" group were also agglutinated by *E. coli* serum O.55 : B.5. A number of O.55 sera gave this reaction. Sera made with *E. coli* O.128 also gave a poor positive slide test with strains of *E. coli* O.55 : B.5. These cross reactions were tested by the tube technique, but heterologous titres only gave trace readings in low dilution. We believe that these findings indicate a very minor antigenic relationship between these serotypes.

#### Biochemical Reactions

Twenty strains representing the six varieties of *Escherichia coli* O.128 were investigated in detail, five non-motile, four of the H.2 variety, three of the H.8 variety, one of the H.9 variety, six of the H.10 variety, and one of the H.12 variety (Table VI). A further 66 strains of the H.2 variety were tested on lactose, glucose, maltose, mannitol, sucrose, salicin, dulcitol, and inositol only, and gave results similar to other H.2 strains. The seven strains of the closely related types having H.10 or H.12 differed from the classical strains in their

TABLE VI  
BIOCHEMICAL REACTIONS OF *E. COLI* O.128

	O.128 : B.12		O.128 : B.12 : H.10	
	"	"	"	"
Lactose	"	H.2	"	H.12
Glucose	"	H.8	"	"
Maltose	"	H.9	"	"
Mannitol	"	"	"	"
Sucrose	AG (1)		AG (1)	
Salicin	AG (1-3)		— (21)	
Dulcitol	AG (1-7)		AG (3)	
Sorbitol	*AG (1-4)		AG (4-10)	
Arabinose	AG (1-3)		AG (1)	
Raffinose	AG (1-2)		AG (1)	
Rhamnose	AG (1-4)		— (21)	
Xylose	AG (1)		†AG (2-3)	
Trehalose	AG (1-3)		AG (1)	
Inositol	AG (1)		AG (1)	
Adonitol	— (21)		— (21)	
Inulin				

— = no change. A = acid. G = gas. Figures in brackets = first true fermentation seen.

\* One strain failed to ferment this sugar.

† H.12 type failed to ferment this sugar.

failure to ferment sucrose and raffinose. All strains produced indole, were V.P. negative, M.R. positive, and failed to utilize citrate, liquefy gelatin, or produce urease. All strains produced H<sub>2</sub>S slowly when tested by the method described by Clarke (1953). This method is very sensitive; in fact, other members of the Enterobacteriaceae described as H<sub>2</sub>S negative will give a positive reaction using Clarke's method.

#### Discussion

This new serotype, *Escherichia coli* O.128 : B.12, has been isolated from sporadic cases and outbreaks of diarrhoea of infants from whom no other accepted pathogen was isolated.

The age group affected by *E. coli* O.128 is similar to that affected by other serotypes of *E. coli*. Most of the cases occurred in babies under 1 year, with a concentration of cases in the 3-6 month age group, whereas after 1 year the numbers diminished. Nevertheless, the oldest child was 2½ years old.

Some strains of *E. coli* O.128 : B.12 had a capsule and produced a slime matrix. Although no detailed investigation of the slime and capsule was made, the tests which were done suggested that the two were either the same or very closely related in their serological reactions. On the few tests done, it seems that the capsular antigen was destroyed by heating to 100° C. for 30 minutes, as the heated suspension was no longer agglutinated by the specific serum. This point is being investigated further. The results for the capsular antigen do not agree with those described by Kauffmann for the capsular antigen of the A type. He found it necessary to autoclave suspensions in order to

destroy the agglutinability of the capsular antigen. We have not encountered a description of *E. coli* having a surface antigen of Kauffmann A type in addition to his B type. Therefore, in this paper, only the symbol C is used to avoid confusion with antigens which may be of a very different nature. Our results agree with those of Wilkinson, Duguid, and Edmunds (1954), who state that, "although differing morphologically, the slime antigens resemble the capsular antigens chemically and serologically." Henriksen (1949) has made a study of some mucoid strains, including *E. coli*, showing capsules. He found that the capsular antigen was not the same in all strains.

Six varieties of *E. coli* O.128:B.12 were found—the non-motile, the H.8, the H.9, the H.10, the H.12, and the most common H.2 varieties. Though few cases of infection due to the non-motile, H.8, H.9, H.10, and H.12 types occurred, there was no difference in the severity of the disease due to these types and the more numerous H.2 type. The H.10 and H.12 types differ slightly from the classical strains in the structure of their somatic and surface antigens, but we believe that the difference is not enough to warrant the use of other symbols, so we shall refer to these as *E. coli* O.128:B.12:H.10 and H.12 respectively. The clinical picture of infection with these varieties was similar to the rest. Type H.10 also caused an institutional outbreak.

### Summary

A new serotype of *Escherichia coli* is described which has been isolated from cases of diarrhoea of infants occurring sporadically and in outbreaks in different parts of England and Scotland.

The age group affected is similar to that found in infantile diarrhoea due to other serotypes of *E. coli*.

This serotype has been accepted as *E. coli* O.128:B.12. Of this there are six varieties, the non-motile, the H.2, which is most common, the H.8, the H.9, and the H.10 and H.12. The H.10 and H.12 varieties we include in this group, though the antigenic structure differs slightly from *E. coli* O.128:B.12. The biochemical reactions of these varieties differ in the failure to ferment sucrose and raffinose.

Some strains produce a capsule and a slime matrix, whose agglutinability seems to be destroyed by heating at 100° C. This finding is still under investigation. The capsular antigen differs from the surface B antigen.

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