

# SEROLOGICAL TYPES OF *ESCHERICHIA COLI* IN ASSOCIATION WITH INFANTILE GASTROENTERITIS

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In an earlier communication (Aboul-Dahab *et al.* 1958) the authors reported on the variability of response to chloromycetin of infants admitted to hospital (August 1954–1955) during a major study on gastroenteritis (Tawil *et al.*, 1959). A relevant feature was the high incidence of relapses among the inpatients of period 3 (May 28, 1955, to August 15, 1955) of that study, particularly the chloromycetin-treated group of cases. The relapses usually coincided with the recovery, from the stools, of strains of *Escherichia coli* that were resistant to chloromycetin. These strains possessed the same somatic O antigen and Dr. F. Ørskov, who kindly checked our findings, identified the strains as belonging to O group 20. Also, *E. coli* was recovered from the stools of many more initial cases. All evidence therefore pointed to the existence of an etiological relation between the *E. coli* and gastroenteritis in the cases reported. Our main concern has been to investigate and report on the antigenic make-up of these strains of *E. coli*. The clinical and epidemiological aspects of the problem have been reported elsewhere (Aboul-Dahab *et al.*, 1958).

## MATERIALS AND METHODS

From rectal swabs taken during the major study only 126 yielded *E. coli* on MacConkey agar when slide agglutinated by a K serum prepared with *E. coli* D48/11054, a strain isolated locally, from the first case of gastroenteritis in the series, and belonging to O group 20. All 126 strains were then submitted to a detailed serological investigation. Their identity and antigenic relation to D48/11054 were established by O and K tube and adsorption tests (Wright and Villanueva, 1953), using the homologous serum as well as the sera prepared against 8 selected strains. The nature of the surface antigen, its O inagglutinability, agglutinin binding capacity, and lability to heat were all tested according to the methods and techniques of Kauffman (1954). All strains were examined

biochemically and the results were recorded after 21 days at 37 C.

*Source of material.* The strains were recovered from 32 infants (28 inpatients and 4 outpatients). Rectal swabs were taken routinely before admission to the hospital, on the second day, weekly or more often during the course of the illness, and on discharge or at postmortem in fatal cases. The distribution of the strains was as follows.

Fifty-three strains were isolated from 10 initial cases that excreted the mentioned coli before admission as well as for some time afterwards.

Sixty-two strains were isolated from 17 cases of gastroenteritis that relapsed in the hospital. Swabs on relapse and thereafter were positive for the type coli.

Three strains were isolated from a healthy ward patient that developed diarrhea.

Four strains were recovered at post-mortem from the gut, bronchopneumonic areas in the lungs, pleural exudate and aural discharge of a fatal case.

Four strains were isolated from four different cases attending the outpatient clinic.

## RESULTS

*Bacteriology.* On MacConkey agar and incubated at 22 and 37 C, all 126 strains produced typical colonies. On blood agar and nutrient agar, the organisms showed a normal smooth colonial morphology without hemolysis.

*Biochemical reactions.* All 126 strains conformed to the biochemical standards of *E. coli* (Kauffmann, 1954). In addition they possessed fermentative features in common in that they readily attacked sorbitol, xylose, and rhamnose in 1 day; trehalose, raffinose, and dulcitol in 1 to 2 days; and salicin and arabinose in less than 10 days. According to their action on sucrose, dulcitol, and raffinose, the strains could be classified into 4 biochemical groups: group 1 (11 strains), sucrose not fermented; group 2

(13 strains), dulcitol not fermented; group 3 (37 strains), sucrose fermented late; group 4 (65 strains), all 3 sugars fermented.

*Serology.* Three antigens were established: a heat stable somatic antigen, a complex B antigen consisting of five heat labile components, and the H antigen.

Using an "O" serum prepared with a heated suspension of *E. coli* strain D48/11054, 38 strains were agglutinated to full titer (12,800), 84 other strains agglutinated to one half titer, and 4 strains agglutinated to one fourth the full titer. Although they varied in their tube-agglutinability by serum D48/11054, all 126 strains proved by a series of adsorption tests to possess a somatic antigen that is identical to that of the homologous strain. Thus the heated suspensions of each of the 126 strains could adsorb completely the "O" agglutinins from antiserum D48/11054. Conversely, strain D48/11054 removed completely the homologous "O" agglutinins from the O antisera of 8 selected strains.

Living suspensions of 126 strains were each agglutinated by an "OK" serum prepared with *E. coli* D48/11054. Fifty-six strains were agglutinated to the titer 160, 39 other strains agglutinated to 1:80, 19 strains agglutinated to 1:40, and the remaining 12 strains to a titer of 1:20. Preliminary information concerning the relationship between the K antigens of these various strains was obtained by a series of adsorption tests, whereby antiserum D48/11054 was adsorbed with each of the 126 strains. From the data in table 1 it may be observed that only 29

TABLE 1

*Agglutination of Escherichia coli strain D48/11054 before and after the adsorption of the homologous OK serum on each of the 126 strains of E. coli O group 20*

Agglutination with Unadsorbed Serum		Agglutination with Adsorbed Serum				
Titer of agglutination	No. of strains in each titer	1:160	1:80	1:40	1:20	0
1:160	56	36	—	—	—	20
1:80	39	30	—	—	—	9
1:40	19	19	—	—	—	—
1:20	12	12	—	—	—	—
Total.....	126	97	—	—	—	29

\* — = No agglutination.

TABLE 2

*Origin and agglutinability of the 8 selected strains by serum Escherichia coli D48/11054*

Strain	Abbreviated Symbol	Titer* of Agglutination	Origin
D48/11054	B	160	The original and initial case of <i>E. coli</i> O group 20 gastroenteritis.
D112/20655	D2	160	Cross infected case.
D122/25655	I	160	Cross infected case.
D115/11755	F1	80	Cross infected case.
D125/29655	K1	80	Cross infected case.
D109/8755	C1	40	Cross infected case.
D122/30655	I4	40	Cross infected case.
D133/3755	N'	20	Initial case of gastroenteritis.
D148/28755	T3	20	Initial case of gastroenteritis.

\* Titers are the reciprocals of the dilutions used.

strains adsorbed completely the K agglutinins from serum D48/11054 whereas the remaining 97 strains failed to do so despite the fact that 66 of the latter were initially agglutinated to one half or to the full titer of the serum. In order to elucidate the exact nature of this serological puzzle, further work was undertaken. For this purpose, 4 pairs, a pair of 2 different strains for each of the 4 titers, were selected from among the group of 97 strains (table 2). An OK serum for each of the 8 selected strains was prepared. All 8 strains as well as strain D48/11054 (designated in the text hereafter as strain B) and the corresponding antisera were then used in cross-agglutination tests. The results are recorded in table 3 and point to the existence of an important antigenic overlap. Thus strains F1, I, D2, and K1 were cross-agglutinated to the titer of each of the homologous antisera, partially so by sera D48 or B, C1, N', and T3, whereas they were not agglutinated by serum I4. Similar results were observed with strains C1 and N', both being cross-agglutinated to the titer of the homologous antisera, partially so by sera D48/11054 or B, F1, I, and T3, whereas they were not agglutinated by sera D2, K1, and I4. Further, it was demonstrated by a series of mirror adsorption tests using the selected strains and their respective

TABLE 3

Cross agglutination between 9 selected strains of *Escherichia coli* O group 20 and their respective antisera

Serum OK	Titer* of Agglutination with Living Suspension of Strains									
	B	C1	N'	F1	D2	K1	I	T3	I4	
D48 or B	160	40	20	80	160	80	160	20	40	
C1	—	640	640	40	40	40	80	80	—	
N'	40	80	80	40	40	40	80	20	40	
F1	40	20	40	640	640	640	640	160	160	
D2	—	—	—	80	80	80	80	—	—	
K1	40	—	—	640	640	640	640	—	40	
I	40	40	—	640	640	640	640	—	—	
T3	—	160	40	80	80	160	80	160	20	
I4	—	—	—	—	—	—	—	—	80	

\* Titers are the reciprocals of the dilutions used.

\* — = No agglutination.

antisera that the F1, D2, K1, and I group of strains possess the same K antigen and constitute a definite *subgroup*, all 4 strains being interchangeable and each adsorbing almost completely the K agglutinins from all 4 sera. After each adsorption, and whichever strain of the subgroup was used for adsorption, the adsorbed serum failed to agglutinate all 4 strains. Similar results were obtained with the C1 and N' group of strains, both being interchangeable and adsorbing completely the agglutinins of either sera and thus forming a different K *subgroup*.

After such an eliminatory sorting out, strains F1 and C1 were each chosen to represent the corresponding subgroup and were compared serologically, in a series of mirror adsorption tests, with the remaining 3 strains in the series B, I4, and T3. Despite the antigenic overlap (table 3), the results of mirror adsorption tests (table 4) have established that each of the 5 strains possessed a different K antigen. A K-specific serum for each of the 5 representative strains was prepared by adsorption on a bacterial deposit of a pool of the different heterologous strains. For instance, specific K subgroup B antiserum was prepared by adsorbing the OK D48/11054 antiserum on a living bacterial deposit of strains F1, C1, I4, and T3. The adsorbed antiserum would then agglutinate strains that are identical to D48/11054 and no other. Similar K specific sera were prepared for subgroups C1, F1, I4, and T3.

*Distribution of the various subgroups.* All 126 strains, initially isolated because of a positive slide reaction with serum D48/11054, were reclassified using the K subgroup specific sera. They occurred with the following frequencies.

Subgroup F1, 33 strains (26.19 per cent);

Subgroup D48/11054 or B, 29 strains (23.01 per cent);

Subgroup C1, 27 strains (21.42 per cent);

Subgroup T3, 25 strains (19.84 per cent);

Subgroup I4, 8 strains (6.34 per cent); and

Not identified, 4 strains (3.17 per cent).

Strains in subgroups C1 were often isolated from the admission swabs of initial cases, whereas the first positive swabs from cross infected cases were often of subgroup F1. Strains in subgroup B were almost equally frequent in all cases and were recovered during the earlier stage of the illness, whereas strains in subgroup T3 were excreted later on and only after the stools had been positive for some other subgroup. Noteworthy is the fact that *E. coli* serotypes O.111, O.55, O.26, and O.86 are distinct from the other cases of gastroenteritis. Those associated with O group 20 often excrete different K types during the course of the illness. The latter were eliminated in succession, one at a time, and never together in a culture made from the same swab. Altogether, 14 of the O group 20 inpatients (50 per cent) excreted 2 or more different K types whereas, the remaining 14 cases (50 per cent) excreted 1 type only.

Because many *E. coli* O group 20 strains when first isolated, and despite repeated subcultures into soft agar, are nonmotile, and because of shortage in diagnostic H-specific antisera, the

TABLE 4

Results of mirror adsorption tests between the 5 representative strains and their corresponding antisera

Titer* of OK Serum before Adsorption	Titer* of Serum after Adsorption on Each of the Strains				
	T3	B	F1	C1	I4
T3 (160)	—	160	160	160	160
B (160)	160	—	160	160	160
F1 (640)	640	640	—	320	640
C1 (640)	160	320	320	—	320
I4 (80)	80	80	80	80	—

\* Titers are the reciprocals of the dilutions used.

investigations concerning the H antigen were not undertaken. F. Ørskov (1956, *personal communication*) who kindly checked our findings, examined 8 strains among which the 5 K representative strains were included. Accordingly:

Strain D48/11054 or B possesses an H antigen type H.34;

Strain D possesses an H antigen type H.34;

Strain C1 could not be grouped with the available antisera;

Strain D1 could not be grouped with the available antisera;

Strain F1 could not be grouped with the available antisera; and

Strain T3 could not be grouped with the available antisera.

The representative strains F1, B, C1, T3, and I4 were O inagglutinable in the living state. Inagglutinability was destroyed by heating the suspensions to 80 C for 1 hr. The heated suspensions were then agglutinated to the titer of the O antiserum. The agglutinogenic capacity of the K antigen was destroyed by heating; a steamed suspension being able to induce the production of O agglutinins only. The agglutinin binding capacity was not significantly diminished by heat; a heated suspension adsorbed all the somatic and most K agglutinins from the homologous antiserum. These tests are regarded as sufficient to classify the K antigen to the B type (Kauffmann, 1954). Thus the K antigen is different from the *E. coli* O group 20 included in the Kauffmann, Knipschildt, and Vahlne (K.K.V.) schema which possesses an L type of K antigen (Kauffmann, 1954).

#### DISCUSSION

Three findings are significant and deserve mention:

1. The present series of 126 cultures of *E. coli* O group 20 differ from the corresponding serotype included in the Kauffmann, Knipschildt, and Vahlne antigenic schema by possessing a different K antigen. Presumably, *E. coli* O group 20 isolated in association with infantile gastroenteritis possess a B variety of K antigen whereas the serologically classified strains possess the L variety (K 17). The occurrence of serologically different forms of K antigen among strains belonging to the same serogroup is not exceptional. *E. coli* O group 8 is one such example

since strains falling in this group may possess a K antigen of the A, B, or even L forms (Kauffmann, 1954). The presence of a B antigen seems apparently to be a distinguishing feature of *Escherichia* serotypes recovered in association with gastroenteritis (Kauffmann, 1951). In this latter respect, *E. coli* O group 20 does not differ from the other enteritis-producing *E. coli* (Charter and Taylor, 1952; Kauffmann and Dupont, 1950; Ørskov, 1951; Ewing *et al.*, 1955) identified so far as: O group 111:B4, 55:B5, 26:B6, 86:B7, and 127:B8 as well as 125:B15 and 126:B16.

2. The B antigen in the present series of *E. coli* O group 20 is complex. Unlike other *E. coli*, the B antigen is not single and homogeneous but consists of a mosaic of different antigenic components. Five components were identified, namely, B or D48, C1, F1, T3, and I4. They were labeled so after the designation of the 5 strains of *E. coli* O group 20 which were used for their identification. In view of the extensive antigenic overlap between the 5 strains as evidenced by their cross-agglutinability to various titers by the corresponding antisera, and since by cross-mirror adsorption tests each strain behaved as if it possessed a specific antigen of its own, it is suggested that the B antigen in each of the 5 strains consists of one prevailing antigen (major) together with a combination of the other (minor).

3. The various B types of *E. coli* O group 20 were often demonstrated in the stools of gastroenteritis patients during the course of the illness. Of the 28 *E. coli* O group 20 cases that were followed in the hospital, each of 14 infants excreted 2 or more B types whereas in the remaining 14 infants 1 type only was demonstrated throughout the disease. Emphasis is laid on the fact that the various types of *E. coli* O group 20 were eliminated at different intervals and that they were never isolated simultaneously in cultures made from the same swab. This latter observation, together with the fact that each of the 5 B types of *E. coli* O group 20 is antigenically stable and does not dissociate into its various components when plated on culture media, point to the possible existence of a process of antigen mutation. This dismisses the possibility of phase dissociation as an explanation of the observed findings.

## SUMMARY

One hundred and twenty-six strains of *Escherichia coli* serogroup O20 were isolated from 32 infants suffering from nonspecific gastroenteritis.

The strains constituted a homogeneous O group, and possessed a B type of K antigen. The B antigen is complex and on analysis yielded 5 different antigenic components. The make-up of the B antigen of any one strain consists apparently of one component which usually predominates (major) whereas the others are present in various combinations as (minor) antigens. They are classified temporarily into 5 serological types according to the major component antigen that characterizes the surface.

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