# INTERRELATIONSHIP OF CERTAIN SHIGELLA AND ESCHERICHIA CULTURES

W. H. EWING, M. C. HUCKS, AND M. W. TAYLOR

*Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Georgia*

Received for publication August 10, 1951

The relationships observed among several serotypes of Enterobacteriaceae make it desirable to discuss the following in one paper:

1. *Shigella dysenteriae* 2 (*Shigella ambigua*).
2. *Escherichia coli* O group 112 a,c of Ewing and Kauffmann (1950) (*Shigella guanabara* of de Assis, 1948).
3. *Escherichia coli*, strain 6182-50 (representative of a new *E. coli* O group, 113).
4. *Escherichia coli* O group 112 a,b of Ewing and Kauffmann (1950).
5. Culture no. 703, a *Shigella*-like bacterium.

de Assis (1948) reported that the cultures which he called *S. guanabara* did not share antigens with any of the known shigellae. Later, de Assis (1950) reported that a heated suspension of one Guanabara culture exhibited a serological relationship to *S. dysenteriae* 2 but that it was possible to distinguish between the two types. The eleven Guanabara cultures (*E. coli* O group 112 a,c) used in this study were received from Dr. de Assis and were the same as those employed in the work reported by Ewing and Kauffmann (1950). The culture of *E. coli* O group 112 a,b (1411-50) was isolated by Dr. H. R. Wolfe from a case of gastroenteritis in a nine-month child and was sent from the Massachusetts State Department of Health to this laboratory for identification. The cultures of *S. dysenteriae* 2 were received for typing and gave typical biochemical and serological reactions. Culture no. 6182-50 was isolated by Dr. J. Olarte from a case of diarrhea in a prematurely born infant in Mexico City. The biochemical reactions given by culture 6182-50 are those of a typical *E. coli* culture. Culture no. 703 was isolated by Dr. H. Gezon of the U. S. Navy Epidemiology Unit 23 in Salerno, Italy, during 1944. Dr. Gezon sent the culture to the senior author in Naples for comparison with other unidentified *Shigella*-like cultures. Culture no. 703 was recovered from feces, but it is not known whether it came from a case or a carrier.

The O antiserums used in this work were prepared with broth cultures that were heated at 100 C for 2½ hours, according to the procedures outlined by Kauffmann (1947, 1951*b*). Suspensions to be used for adsorption of agglutinin from O antiserums were heated at 100 C for 1 hour. Broth culture O antigens used in agglutination tests were also heated at 100 C for 1 hour. Antiserums for the flagellar (H) and thermolabile K antigens of the bacteria were prepared after the methods given by Kauffmann (1947, 1951*a*), and tests with the antiserums were performed in the manner advised by that investigator. For other references

on the serological procedures used for the determination of O, K, and H antigens, see Kauffmann (1943, 1944, 1947), Knipschildt (1945), and Vahlne (1945).

*Shigella dysenteriae 2 and Escherichia coli O group 112 a,c.* The relationship of *E. coli* O group 112 a,b (1411-50) and *E. coli* O group 112 a,c (Guanabara) was established by Ewing and Kauffmann (1950). The two share a common factor, a, but each contains an unrelated antigen, labeled respectively b and c.

TABLE 1

*The O antigenic relationships of Shigella dysenteriae 2, a Shigella-like culture (703), and Escherichia coli cultures*

| O ANTIGEN SUSPENSIONS (100 C, 1 HR) | O ANTISERUMS | | | | | | |
|---|---|---|---|---|---|---|---|
| | Unabsorbed | *Escherichia coli* O group 112 a,b | | | | *Escherichia coli* O group 112 a,c | |
| | | Adsorbed by | | | Unabsorbed | Adsorbed by | |
| | | *S. dysenteriae* 2 | 6182-50 | *E. coli* O group 112 a,c | | *E. coli* O group 112 a,b | *S. dysenteriae* 2 |
| *Shigella dysenteriae* 2 | 640* | 0† | | 0 | 10,240 | 10,240 | 0 |
| *Escherichia coli* O group 112 a,b (1411-50) | 20,480 | 10,240 | 5,120 | 5,120 | 2,560 | 0 | 0 |
| 703, *Shigella*-like | 10,240 | | 5,120 | | 640 | | |
| *E. coli* O group 112 a,c (712-50, 1685) | 20,480 | 0 | 5,120 | 0 | 20,480 | 20,480 | 0 |
| *E. coli* 6182-50 | 1,280 | 320 | 0 | 320 | 0 | | |

| | O ANTISERUMS | | | | |
|---|---|---|---|---|---|
| | Unabsorbed | *Shigella dysenteriae* 2 | | | *Escherichia coli* 6182-50 |
| | | Adsorbed by | | Unabsorbed | Adsorbed by |
| | | *E. coli* O group 112 a,b | *E. coli* O group 112 a,c | | *E. coli* O group 112 a,b |
| *S. dysenteriae* 2 | 20,480 | 5,120 | 0 | 0 | |
| *E. coli* O group 112 a,b (1411-50) | 160 | 0 | 0 | 640 | 0 |
| 703, *Shigella*-like | 640 | 0 | | 640 | 0 |
| *E. coli* O group 112 a,c | 10,240 | 5,120 | 0 | 0 | 0 |
| *E. coli* 6182-50 | 0 | | | 20,480 | 10,240 |

* Figures indicate the reciprocal of the highest dilution that gave visible agglutination.
† 0 equals negative in dilutions of 1:40 and higher.

When O antigen suspensions of the eleven *E. coli* O group 112 a,c cultures were tested in *S. dysenteriae* 2 antiserum, all were agglutinated to the homologous titer of the antiserum. Also, cultures of *S. dysenteriae* 2 reacted to the titer of an O antiserum prepared with *E. coli* O group 112 a,c. The results of reciprocal adsorption tests (table 1) indicate that the thermostable somatic antigens of *S. dysenteriae* 2 and *E. coli* O group 112 a,c are identical. Each removes all agglutinin from antiserum prepared with the other. Suspensions of *S. dysenteriae* 2 cultures were agglutinated in O antiserum for *E. coli* O group 112 a,b in a

dilution of 1:640, and heated antigens of *E. coli* O group 112 a,b reacted in a similar dilution of *S. dysenteriae* 2 antiserum. Reciprocal adsorption tests indicate (table 1) that the cross agglutination of *S. dysenteriae* 2 and *E. coli* O group 112 a,b is caused by the factor a, common to both bacteria. *E. coli* O group a,b antiserum adsorbed with a suspension of *S. dysenteriae* 2 no longer agglutinated the *S. dysenteriae* 2 or Guanabara cultures (*E. coli* O group 112 a,c), while the titer of the antiserum for *E. coli* O group 112 a,b was reduced only slightly (1:20,480 to 1:5,120). Conversely, the agglutinative titer of *S. dysenteriae* 2 antiserum was reduced only slightly when adsorbed by *E. coli* O group 112 a,b. This antiserum no longer reacted with suspensions of the adsorbing culture but reacted strongly with *S. dysenteriae* 2 and Guanabara cultures.

*E. coli O group 112 a,b and E. coli, strain 6182-50 (O group 113).*[1] Suspensions of *E. coli*, cultures 6182-50 and O group 112 a,b (1411-50), cross react in dilutions of 1:1,280 and 1:640, respectively, in O antiserums prepared with the two types

TABLE 2

*The relationship of Escherichia coli O group 112 a,b and a Shigella-like culture (703)*

| O ANTIGEN SUSPENSIONS (100 C, 1 HR) | O ANTISERUMS | | | |
|---|---|---|---|---|
| | 703 | | O group 112 a,b | |
| | Unadsorbed | Adsorbed by O group 112 a,b | Unabsorbed | Adsorbed by 703 |
| Culture no. 703 (*Shigella*-like) | 2,560 | 0 | 10,240 | 0 |
| *E. coli* O group 112 a,b | 2,560 | 0 | 20,480 | 0 |

(table 1). The results of reciprocal adsorption tests with these antiserums indicate that culture 6182-50 contains a portion of the b factor of *E. coli* O group 112 a,b. Ancillary evidence that this culture contains a portion of the b factor is afforded by the fact that culture 6182-50 continued to react in a dilution of 1:320 in O group 112 a,b antiserum adsorbed by *S. dysenteriae* 2. However, *E. coli*, culture 6182-50, contains a major O antigen that is not related to *E. coli* a,b or to any other described *E. coli* O group. Antigens prepared with *E. coli*, culture 6182-50, did not react in unadsorbed *S. dysenteriae* 2 antiserum, and this culture bears no O antigenic relationship to *S. dysenteriae* 2, nor to *E. coli* O group 112 a,c.

*A Shigella-like culture (703 and E. coli O group 112 a,b).* The biochemical reactions of culture no. 703 are as follows: Acid is produced without gas formation from glucose, mannitol, and sorbitol within 24 hours' incubation. Arabinose is fermented after 5 days' incubation. Acid is not produced from lactose, sucrose, salicin, maltose, dulcitol, rhamnose, inositol, xylose, or adonitol. The bacteria are nonmotile, do not hydrolyze urea, nor form acetylmethylcarbinol. Indole is produced, and the methyl red test is positive. Culture 703 does not grow on

[1] Kauffmann (1951c) reports that culture 6182-50 contains O antigens identical with those of Wramby's W32 which was isolated from a calf. These cultures W32 and 6182-50 constitute a new *E. coli* O group, O113.

Simmons' citrate agar nor does it give a positive reaction on the citrate medium of Christensen (1949). Except for the relationship to *S. dysenteriae* 2 (table 1), type 703 bears no serological relationship to recognized members of the *Shigella* group and is regarded as a serotype that should be given consideration for possible future addition to the *Shigella boydii* group. The relationship of culture no. 703 to *E. coli* O group 112 a,b is shown in table 2. The two types cross agglutinate to the titers of their respective antiserums, and reciprocal adsorption tests indicate that the heat stable somatic antigens of the two cultures are identi-

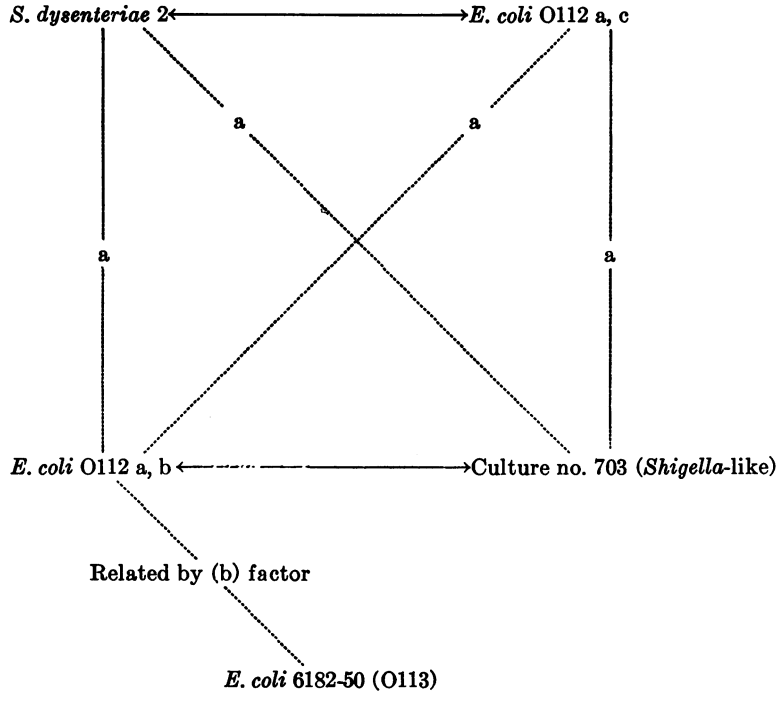


*Figure 1.* O antigen interrelationships. Arrows indicate identical O antigens. Dotted lines represent relationship by the factors indicated.

cal. The O antigen relationships previously described are shown diagrammatically in figure 1.

The flagellar (H) antigens of cultures of *E. coli* O group 112 a,b (1411-50) and 6182-50 (O113) were determined by testing broth cultures in each of the 22 *E. coli* H antiserums. These tests indicate that *E. coli* O group 112 a,b contains H antigen 18 and that the flagellar antigens of culture 6182-50 (O113) are the same as H 21 of the *E. coli* antigenic schema.

Studies on the K antigens of all of the foregoing cultures were instituted but are not completed. However, the results indicate that *E. coli* 6182-50 (O113) possesses a K antigen and that cultures of *S. dysenteriae* 2, *E. coli* O group 112

a,b, and O group 112 a,c also contain K antigens. O agglutination is inhibited when living antigens of these cultures containing K antigen are tested in O antiserums. It seems clear that the K antigen of *S. dysenteriae* 2 and *E. coli* O group 112 a,c cultures is the same or very closely related while *E. coli* O group 112 a,b contains a different K antigen. Whether or not the K antigens of these cultures can be identified as one of the 59 known K antigens has not been determined. None of the preceding cultures contain alpha antigen. A study of the occurrence in the *Shigella* group of thermolabile antigens of the K type is in progress, and further work on these cultures will be included in it.

## DISCUSSION

The results indicate clearly that the O antigens of *S. dysenteriae* 2 and *E. coli* O group 112 a,c are identical. Other examples of such relationships are known. Wheeler and Stuart (1946) reported the relationship of three coliform organisms that were antigenically related to *S. dysenteriae* 3 (Q771). The number of these related coliform cultures has been extended to six (authors' unpublished data). Veazie (1950) reported that a very close relationship was found between *S. boydii* 4 (P274), *S. boydii* 5 (P143), and *E. coli* O53 and O79, respectively. The writers confirmed this report and found that the O antigens of *S. boydii* 4 are identical with those of *E. coli* O53, and the O antigens of *S. boydii* 5 are identical with those of *E. coli* O79 as demonstrated by reciprocal agglutinin adsorption tests. These observations were also confirmed by Kauffmann (1950). Odden (1951) found that the O antigens of *S. dysenteriae* 5 (Q1030) and *E. coli* O58 are identical and that those of *S. boydii* 2 and *E. coli* O87 are strongly related but are not identical. These observations were confirmed in this laboratory. Knowledge of relationships such as those outlined before is of the utmost importance in diagnostic work. Serological studies on enteric microorganisms must be as complete as possible and must always be confirmed by a study of the biochemical characteristics. There are many other reports in the literature on the subject of interrelationships among members of the family Enterobacteriaceae, and workers in the field of enteric bacteriology now regard the family as one large interrelated group within which there are islands of dense population which are not sharply demarcated. These islands correspond to the various arbitrary groups such as *Salmonella*, *Shigella*, Alkalescens-Dispar, etc. A view of the enteric group similar to this is also taken by Borman, Stuart, and Wheeler (1944), the Enterobacteriaceae Subcommittee (1951), Kauffmann (1951*b*), and by other investigators.

Sound work on the relationships of the various bacterial types included in the family Enterobacteriaceae was made possible by the clarification of the serology of the *E. coli* group by Kauffmann and his collaborators. It is now possible to elucidate many relationships which, hithertofore, were not entirely clear. As an example of this, Ferguson and Wheeler (1946) described two paracolon bacteria that are related through minor antigens to certain *Shigella flexneri* serotypes and to *S. boydii* 5. These two paracolon cultures are now known to belong to *E. coli* O group 4 and that the antigenic factors involved in the rela-

tionships described by Ferguson and Wheeler are the same factors that relate some members of Alkalescens-Dispar group to *S. flexneri* and *S. boydii* 5 cultures. Also (authors' unpublished data) many paracolon bacteria similar to *Escherichia* which formerly could not be classified serologically now can be placed in one or another of the *E. coli* O groups.

It is proposed that culture 703 should be considered for possible future addition to the *S. boydii* group. Group C of the *Shigella* schema now consists of seven serotypes (see the Enterobacteriaceae Subcommittee Report, 1951, or Ewing, 1949). Courtois and Vandepitte (1950) suggested that type 112, described by Cox and Wallace (1948), should be added to the *S. boydii* group. The Shigella Commission of the Enterobacteriaceae Subcommittee has accepted type 112 as provisional *S. boydii* 8. Descriptions of provisional *S. boydii* 9, 10, and 11 are in preparation.

SUMMARY

The antigenic relationship of *Shigella dysenteriae* 2 (*Shigella ambigua*) and *Escherichia coli* O group 112 a,c is reported. The O antigens of these two bacterial types are identical as demonstrated by reciprocal agglutinin adsorption tests.

Further studies on *E. coli* O group 112 a,b are reported, and a *Shigella*-like culture (703) that contains thermostable somatic antigens identical with it is described.

Preliminary investigations of a new *E. coli* O group are outlined.

A brief discussion of interrelationships among enteric bacteria is included, and the importance of such relationships to diagnostic bacteriology is stressed.

REFERENCES

BORMAN, E. K., STUART, C. A., AND WHEELER, K. M. 1944 Taxonomy of the family Enterobacteriaceae. J. Bact., **48**, 351–367.

CHRISTENSEN, W. B. 1949 Hydrogen sulfide production and citrate utilization in the differentiation of the enteric pathogens and the coliform bacteria. Research Bull., Weld Co., Colorado Health Dept., **1**, 3–16.

COURTOIS, G., AND VANDEPITTE, J. 1950 Un noveau serotype de *Shigella* paradysenteriae type 112 de Cox et Wallace. Ann. Soc. Belge de Med. Trop., **30**, 149–154.

COX, C. D., AND WALLACE, G. I. 1948 A study of *Shigella* isolated in India and Burma, with special reference to two previously undescribed serotypes. J. Immunol., **60**, 465–473.

DE ASSIS, A. 1948 *Shigella guanabara*, tipo serológico destacado do grupo do *B. ceylonensis-dispar*. O Hospital, **38**, 505–518.

DE ASSIS, A. 1950 Personal communication.

ENTEROBACTERIACEAE SUBCOMMITTEE 1951 Report of proceedings. Fifth International Congress for Microbiology. *In press*.

EWING, W. H. 1949 *Shigella* nomenclature. J. Bact., **57**, 633–638.

EWING, W. H., AND KAUFFMANN, F. 1950 A new coli O-antigen group. U. S. Pub. Health Repts., **65**, 1341–1343.

FERGUSON, W. W., AND WHEELER, W. E. 1946 Two paracolon cultures related antigenically to *Shigella paradysenteriae*. J. Bact., **51**, 107–112.

KAUFFMANN, F. 1943 Über neue thermolabile Körperantigene der Coli-Bakterien. Acta Path. Microbiol. Scand., **20**, 21–44.

KAUFFMANN, F. 1944 Zur Serologie der Coli-Gruppe. Acta Path. Microbiol. Scand., **21**, 20–45.

KAUFFMANN, F. 1947 The serology of the coli group. J. Immunol., **57**, 71–100.

KAUFFMANN, F. 1950 Personal communication.

KAUFFMANN, F. 1951*a* The differentiation of *Escherichia* and *Klebsiella* types. C. C. Thomas, Springfield, Illinois.

KAUFFMANN, F. 1951*b* Enterobacteriaceae. Ejnar Munksgaard, Nørregade 6, Copenhagen, K, Denmark.

KAUFFMANN, F. 1951*c* Personal communication.

KNIPSCHILDT, H. E. 1945 Undersølgelser over coligruppens serologi med saerligt henblik paa kapselformerne. Nyt. Nordisk Forlag, Arnold Busck, Copenhagen.

ODDEN, C. 1951 Acta Path. Microbiol. Scand. *In press.*

VAHLNE, G. 1945 Serological typing of colon bacteria. Gleerupska Univ., Bokhandeln, Lund.

VEAZIE, L. 1950 Antigenic identity of *Shigella alkalescens* type I and Kauffmann's *Escherichia coli* O group 1. Proc. Soc. Exptl. Biol. Med., **74**, 350–352.

WHEELER, K. M., AND STUART, C. A. 1946 The mannitol-negative *Shigella* group. J. Bact., **51**, 317–325.