DETECTION OF A NEW SEROTYPE OF ESCHERICHIA COLI, E. COLI 0127: B8, ASSOCIATED WITH ACUTE DIARRHEA IN INFANTS\(^1\)

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In 1927 Adam reported the association of certain fermentative types of *Escherichia coli* with diarrhea in infants. Goldschmidt (1933) made similar observations. Bray (1945) in England demonstrated the presence of a specific serologic “O” type, O111, of *E. coli* in an epidemic of diarrhea among newborn infants and focused attention on *E. coli* as a possible etiologic agent. Subsequent studies were made in England by Giles and Sangster (1948); Giles, Sangster, and Smith (1949); Smith (1949); Taylor, Powell, and Wright (1949); Taylor and Charter (1952); Charter and Taylor (1952); Smith, Galloway, and Speirs (1950); Rogers (1951); Rogers and Koehler (1951); Alexander et al. (1952); Wright and Villanueva (1953); Wright and Roden (1953); and in Scotland by Shanks and Studzinski (1952) who demonstrated two serologic “O” types, O111 and O55 according to the classification of Kauffmann. These two particular types of *E. coli* were found predominating in the stools of infants with epidemic diarrhea and were found infrequently in stool cultures from other infants. Similar findings have been made in Germany by Beeuwkes, Hodenplij, and ten Seldam (1949); Braun (1950, 1951); Laurell et al. (1951); Krepler and Zischka (1952); Zischka (1952); Opitz (1952); and Hoster (1953); in France by Buttiaux et al. (1951) and Clement et al. (1953); in Denmark by Kauffmann and Dupont (1950) and Dupont (1951); in Jerusalem by Drimmer-Herrnheiser and Olitzi (1951); in Australia by Williams (1951); in Israel by Rappaport and Henig (1952); in Mexico by Olarte and Varela (1952); and in Ceylon by Schmid and Velaudaspillai (1952). In the United States, bacteriological and clinical studies with certain serotypes of *E. coli* associated with diarrhea in infants date from 1950, and have been made, until recently, primarily in the laboratories of Doctor Erwin Neter and Doctor William W. Ferguson: Neter and Shumway (1950); Neter et al. (1951, 1952a,b, 1953a,b); Neter and Webb (1951); Gorszynski and Neter (1953); Neter and Zalewski (1953); Ferguson, Jennings, and Gottshall (1951); Ferguson and June (1952); Modica, Ferguson, and Ducey (1952); and June, Ferguson, and Worfel (1953).

We shall present at this time the results of our bacteriological study of an epidemic of diarrhea among infants on two wards of the Children's Hospital, Cincinnati, Ohio, in which a new serotype of *E. coli*, *E. coli* O127: B8, was detected. The results of our clinical study (Cooper, Walters, Keller, Sutherland and Wiseman, 1955) will be presented elsewhere.

Between late November, 1953, and March, 1954, there occurred on our two infants' wards an unusual incidence of diarrhea which progressed and spread regardless of the use therapeutically of various antibiotics and sulfonamide. The fact that the majority of the patients were infants, some newborn premature and some newborn full term, and that repeated bacteriological study of rectal swab specimens failed to reveal the presence of *Shigella* or *Salmonella* led us to consider the possibility that we were encountering an epidemic of diarrhea of the newborn possibly due to one of the specific serotypes of *E. coli* previously found to be associated with diarrhea. The epidemic aspect was emphasized by the fact that a few infants admitted for some condition other than diarrhea developed the same diarrheal disease a few days after admission. Prior to the time of realization of the presence of an epidemic, rectal swabs were obtained for culture only from patients with diarrhea; thereafter rectal swabs were
obtained from all infants on the two involved wards regardless of the presence or absence of diarrhea, also from all attending personnel in order to detect inapparent cases and carriers.

**METHODS**

Stool specimens were obtained by using rectal swabs which were placed immediately in test tubes containing 2.0 ml of sterile glycerin preservative solution (Connecticut State Department of Health, 1945). These swabs were cultured on sorbitol agar, MacConkey agar (Difco), and blood agar prepared with tryptose soy agar (Baltimore Biological Laboratory, Baltimore, Maryland) plus 5 per cent fresh defibrinated rabbit blood. In addition the same swabs were cultured in the Bacteriology Laboratory of the Children's Hospital for *Shigella* and *Salmonella* on SS agar (Difco), MacConkey agar, brilliant green agar (Difco), and in tetraionate broth (Difco) base which, after incubation overnight, was subcultured onto the other three media. The cultures studied in the Bacteriology Laboratory of the Hospital failed with few exceptions to reveal the presence of any intestinal pathogen.

The cultures studied in our laboratory revealed, in acutely ill patients, a predominance of a type of *E. coli* differing from the *E. coli* usually found in that they failed to ferment sorbitol, were weak producers of indole, and did produce colonies on sorbitol agar resembling colonies of *E. coli O111* and O55, but were not agglutinated in antisera (kindly supplied by Dr. W. H. Ewing) specific for these two types of pathogenic *E. coli*. As the patients convalesced this unusual type of *E. coli* gradually decreased in numbers, and the normal coliform bacteria became predominant.

It soon became evident that the sorbitol agar was much superior to blood agar and MacConkey agar for recognition of colonies of this unusual *E. coli*, and as the number of specimens increased, we began to rely entirely on the sorbitol agar for detection of their colonies. This medium is prepared by the Difco Laboratories with some modifications of the formula of Rappaport and Henig (1962) who substituted d-sorbitol for lactose in MacConkey agar, thus taking advantage of the fact that *E. coli O111* and O55 ferment sorbitol late or not at all. Since the strain of *E.

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We are indebted to Dr. C. W. Christensen of the Difco Laboratories, Detroit, Michigan, for supplying us generously with this medium.
with production of acid and gas. Salicin and inositol were not fermented in 30 days. Urease was not produced. Thirty strains did not ferment sorbitol in 30 days; 14 strains produced acid and a small amount of gas after 13 to 25 days.

Serological antigenic classification of the strains of E. coli isolated from our patients was made possible through the courtesy of Dr. W. H. Ewing (Communicable Disease Center, Federal Health Service, Federal Security Agency, Chamblee, Georgia). Early in the study the strains of E. coli which we were detecting and which did not agglutinate in antisera specific for E. coli O111 and O55 were forwarded to Dr. Ewing for serological study. It was Dr. Ewing's conclusion (personal communication) that our E. coli was a particular serotype with a new "O" antigenic group which most likely would be designated by Dr. F. Kauffmann (1954, personal communication) in Denmark as O127. Doctor Kauffmann's subsequent study confirmed the earlier opinion of Ewing.

Diagnostic antisera. Antisera were used for identification of strains of three serotypes of E. coli isolated in this study: E. coli O127 antisera prepared in our laboratory and E. coli O111 and O55 antisera. The E. coli O127 antisera was prepared by injecting rabbits with a suspension of the first strain isolated from a fatal case (C.H.). The rabbits were injected first with a killed suspension heated in a boiling water bath for 1½ hours to remove the "B" envelope antigen, thus exposing the "O" antigen, and then injected with the living suspension to stimulate "B" antibody response. These suspensions were standardized so as to permit 30 per cent light transmission with filter 655 in a Coleman nephocolorimeter, Model 9. This antisera had an agglutinin titer for the "O" antigen of 12,800 and reacted with the "B" antigen by slide agglutination at a dilution of 200. Homologous types of E. coli isolated from other patients were agglutinated to the maximum "O" titer, and "B" agglutination occurred immediately in dilution 1:10. Strains of E. coli O111 and O55 and strains of E. coli commonly found in stool cultures were not agglutinated. "B" agglutinability was determined by suspending a small amount of young surface growth from a colony or sorbitol iron agar slant culture in a loopful of antisera diluted 1 to 10 on a glass slide. If positive, agglutination occurred almost immediately. Then "O" agglutina-

bility was determined. Detection of "O" antigen by agglutination was made by adding a standardized suspension to test tubes containing serial dilutions of diagnostic antiserum, 200 to 12,800 inclusive, and by incubating 18 hours in a water bath at 55 C. The titer of the serum was read as the highest dilution showing agglutination. The saline suspensions for detection of "O" antigen were freshly prepared by washing the growth from agar slants and heating for two hours in a boiling water bath to remove the "B" envelope-interfering-antigen and to expose the "O" somatic antigen. The antigens for agglutination were standardized so as to permit 85 per cent light transmission when read in a Coleman nephochromometer, Model 9 with filter 655. A buffered saline control permitted 100 per cent light transmission. In addition Dr. Ewing made antigenic studies of cultures of E. coli O127 from each of the 44 patients, confirmed our serologic findings that they were the O127 somatic "O" type and did contain the K envelope antigen "B 8", thus designating this serotype as E. coli. O127: B 8.

Results

Rectal swab cultures on a total of 158 infant patients and 52 adult personnel were studied for the presence of certain serotypes of E. coli associated with diarrhea and other intestinal pathogens between late November, 1953, and March, 1954. E. coli O127 was isolated from 44 infant patients and one nurse in attendance on infants' wards; E. coli O111 from 6 patients; Shigella from 2 patients; Salmonella from 5 patients and one student nurse; E. coli O127 from one infant who had previous rectal swab cultures positive for E. coli O111; and both E. coli O127 and Salmonella from one patient. The types of Shigella isolated were Shigella flexneri 2 and Shigella alakelescens. The types of Salmonella isolated were S. brendeney, S. melagrdis, S. oranienburg, S. saint paul, S. tennessene, and S. typhimurium.

Sensitivity tests. Early in this study sensitivity disks (Difco) were used to obtain rapid general information regarding the sensitivity of these strains to antibiotics and sulfadiazine. Twenty-four strains of E. coli O127 were tested with the disk method using two concentrations, 10 and 30 μg of antibiotics and 10 and 50 μg of sodium sulfadiazine. All strains were found by this method to be sensitive to neomycin, aureomycin, chloramphenicol, and terramycin and resistant.
to streptomycin and sodium sulfadiazine. Later the tube dilution method was used for determining end points of sensitivity. These data will be included in another publication (Cooper, Walters, Keller, Sutherland and Wiseman, 1955) setting forth the critical aspects of this study.

SUMMARY

Bacteriological study of an epidemic of diarrhea among infants in the Children's Hospital, Cincinnati, Ohio, revealed the presence of a new serotype of E. coli, identified as E. coli O127: B 8.

A total of 158 infant patients and 82 adult personnel were cultured for the presence of certain serotypes of E. coli associated with diarrhea in infants and other intestinal pathogens during a four month period. E. coli O127 was isolated from 44 infant patients on the two infants' wards and from one apparently healthy nurse in attendance on these wards. This type of E. coli was found in practically pure culture in rectal swab cultures from infants in the acute phase of their disease.

The details of biochemical culture reactions and serological studies are presented.

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