ESCHERICHIA COLI O111a,111c:B4. A NEW SEROTYPE ISOLATED FROM MONKEYS

W. H. EWING, M. M. GALTON, AND K. E. TANNER

Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia

Received for publication October 25, 1954

The purpose of this paper is to present the results of biochemical and serological studies made with a group of *Escherichia coli* cultures isolated from monkeys at the Okatie Farms near Bluffton, South Carolina. These *E. coli* strains were recovered on SS agar from rectal swab specimens collected during an epizootic of enteric disease in a colony of about 4,000 *Macaca mulatta* and *Macaca irus*. Details of the epizootic and a full report of the results of bacteriological and parasitological examinations will be published.[1] The epizootic was serious with a high mortality rate, and it seemed probable that most of the illness was caused by the several *Shigella* and *Salmonella* serotypes that were isolated. During this outbreak, 99 cultures of *E. coli* were recovered which were investigated further because they were agglutinated by antiserum prepared with *E. coli* serotype O111:B4. Each of the 99 cultures was from a separate monkey, as follows: Twenty-three cultures from monkeys hospitalized for respiratory infection; no diarrhea. Seventy-five cultures from monkeys with diarrhea, hospitalized. *Salmonella* or *Shigella* serotypes also were recovered from 11 of these animals. One culture from a monkey in the "Well Area".

Sixty-four of the strains of the serotype related to *E. coli* O111 were isolated from monkeys hospitalized for diarrhea, and salmonellae or shigellae were not recovered from these animals. However, it is not the purpose in this paper to attempt assessment of the possible pathogenic propensities of the new serotype. Fifty-eight of the above mentioned cultures were sent to these writers for further investigation. These were received in three shipments, in May, June, and July, 1954.

Biochemical reactions. The biochemical reactions given by the 58 *E. coli* cultures from monkeys were similar and constitute a new biotype within O group 111. Glucose, mannitol, sucrose, and sorbitol were fermented within 24 hours' incubation. Only two cultures produced gas from glucose, but the majority of strains formed small gas volumes (bubble) from mannitol, sucrose, and sorbitol. Lactose was fermented, without gas formation, after 3 to 5 days' incubation. Salicin was fermented, usually with the formation of a small bubble of gas, but in several instances the reaction was delayed. Acid was not produced from adonitol or inositol during 30 days' incubation, growth did not occur on Simmons' citrate agar, urea was not hydrolyzed, the Voges-Proskauer reaction was negative, hydrogen sulfide was not formed in triple sugar iron agar, and the cultures were nonmotile. The methyl red reaction was positive, and indole was formed. Several of the cultures were plated on MacConkey's agar, and these gave rise either to a mixture of colonies, predominately colorless with a few pink colonies, or to colorless colonies only which developed pink papillae upon further incubation. Lactose was fermented within 24 hours upon transfer of pink colonies or of papillae to lactose broth.

Serological studies. Living suspensions of the 58 *E. coli* cultures isolated from monkeys were agglutinated by *E. coli* O111:B4 antiserum in slide tests. When tested in O antiserum for *E. coli* O group 111, the same suspensions were inagglutinable or were agglutinated only slightly, indicating the presence of K antigen related to B4. Upon titration, unheated suspensions of all the strains were agglutinated to the titer (1:320) of B4 antiserum. A K antiserum was prepared with one of the cultures, 932-54, and reciprocal

[1] This report will emanate from the laboratories of the Florida State Board of Health, where the described cultures were first isolated and studied. The investigations made at the Florida State Board of Health laboratories were conducted with the joint support of the National Foundation for Infantile Paralysis and the Armed Forces Epidemiological Board.

TABLE 1

The relationship of the O and B antigens of Escherichia coli O group 111 cultures from infantile diarrhea and related cultures from monkeys

E. coli Antigen Suspensions	*E. coli* OB Antisera			
	O111:B4 (585-52)		OB 932-54	
	Unabsorbed	Absorbed by 932-54 (100 C)	Unabsorbed	Absorbed by 585-52 (100 C)
O111:B4 (585-52), living*	320	0	640	0
O111:B4 (585-52), 100 C	5,120	1,280	5,120	0
932-54, living*	320	0	640	0
932-54, 100 C	5,120	0	5,120	320

* The living cultures used in these tests were inagglutinable in O antisera in both slide and tube tests.

agglutinin adsorption tests were made with this and *E. coli* O111:B4 antiserum (585-52). The results of these tests (table 1) indicated that the K antigen of culture 932-54, and the other strains recovered from monkeys, was identical with K antigen B4. Ancillary evidence that this K antigen was of the B variety was afforded by agglutinin adsorption experiments in which K antiserum 932-54 was adsorbed with a suspension of the homologous culture that had been heated at 100 C for 1 hour. Since both O and K agglutinins were removed from the antiserum by the heated suspension, it was clear that the agglutinin binding power of the K antigen was not destroyed and, hence, that the K antigen was of the B variety.

When tested in serial dilutions of O antiserum for *E. coli* O group 111 (585-52), O antigen suspensions of the 58 strains from monkeys all were agglutinated at 1:2,560 to 1:5,120 (homologous titer, 1:10,240). An O antiserum was prepared with culture 932-54 which agglutinated O antigen suspensions of all of the cultures recovered from monkeys in dilutions of 1:5,120 to 1:10,240. O antigen suspensions of cultures of *E. coli* O111 from cases of infantile diarrhea were agglutinated in dilutions of 1:640 to 1:1,280 in O-932-54 antiserum. When antiserum O-932-54 was absorbed by *E. coli* O111, no. 585-52, all agglutinin for the absorbing strain was removed, and although the homologous titer was reduced somewhat, culture 932-54 and other strains from monkeys continued to react in dilutions of 1:1,280. In the reciprocal adsorption tests, the titer for the homologous *E. coli* O111 culture, no. 585-82, was similarly reduced, but a specific factor remained in the O antiserum for *E. coli* O111 cultures. It may be concluded that the O antigens of the *E. coli* strains isolated from monkeys were related to those of *E. coli* O111 cultures recovered from cases of infantile diarrhea but were not identical. Arbitrary formulas for the two *E. coli* serotypes may be written as follows: O111a,111b:B4 infantile diarrhea strains; O111a,111c:B4 cultures from monkeys.

In slide tests in which cross adsorbed O antisera were employed, all 58 strains from monkeys reacted only in factor O111c serum while 25 cultures of *E. coli* O111 cultures from cases of infantile diarrhea agglutinated only in antiserum for factor O111b. Thus, slide tests with properly adsorbed antisera may be used to differentiate these two serotypes.

The O antigenic relationships mentioned above were confirmed in other agglutinin adsorption studies in which OB antisera prepared with cultures 932-54 and *E. coli* O111:B4 (585-52) were employed. The results are given in table 1, along with the results of studies on the B antigen.

Comment. The described antigenic differences between *E. coli* O group 111 cultures isolated from cases of infantile diarrhea and those recovered from monkeys at Okatie Farms illustrate the importance of detailed analysis of strains which appear to belong to the same serotype, especially when such strains are from diverse sources. Recently, antigenic differences of a somewhat similar nature were reported by Ewing *et al.* (1954, *in press*) in the O and B antigens of *E. coli* O86a:B7 and O86a,86b:B9:H36 cultures and between those of O127a:B8 and O127a,127b:B10:H4 strains. In these instances, the *E. coli* O86a and O127a cultures all were recovered from cases of diarrhea of the newborn while the O86a,86b and O127a,127b strains all were from the stools of adults or children who did not have symptoms of intestinal disease. Ørskov (1954*a*) demonstrated that a group of *E. coli* O group 86 cultures from bovine mastitis contained a K antigen which differed from the K antigens found in strains from cases of infantile diarrhea. Also, Ørskov

(1954*b*) described a culture of *E. coli* O group 111 which differed from the usual in that it contained an additional O antigenic factor. This strain was the only microorganism of significance recovered from the stools of an adult ill with severe diarrhea. Although such antigenic differences may appear to be unimportant, it is felt that they, and others like them, may prove to be of considerable value in epidemiological studies for the differentiation of *E. coli* serotypes isolated from disease processes in humans and those recovered from normal humans or from animals.

SUMMARY

The results of biochemical and serological studies on 58 cultures of a new *Escherichia coli* serotype isolated from monkeys were reported. The O antigens of the new serotype were related to those of *E. coli* O111 cultures recovered from cases of diarrhea of the newborn but were not identical with them. The formula for the latter cultures may be written O111a,111b:B4 and that for the former, O111a,111c:B4.

These findings provided an excellent example of the value of intimate serological study in the differentiation of serologically related strains which are epidemiologically distinct. They also called attention to the necessity of careful examination of the antigenic properties of "infantile diarrhea types" isolated from diverse sources.

REFERENCES

ØRSKOV, F. 1954*a* Studies on a number of *Escherichia coli* strains belonging to O group 86. Acta Pathol. Microbiol. Scand., **35,** 179–186.

ØRSKOV, F. 1954*b* A new *Escherichia coli* type belonging to O-group 111 (111+:B4:11). Acta Pathol. Microbiol. Scand., **35,** 72–74.