E. coli Serotype D433: Occurrence in Intestinal and Respiratory Tracts, Cultural Characteristics, Pathogenicity, Sensitivity to Antibiotics.* (18246)

ERWIN NETER AND CLARE N. SHUMWAY.

From Departments of Bacteriology and Pediatrics of the Children's Hospital and University of Buffalo, School of Medicine, Buffalo, N. Y.

Although progress has been made in the knowledge of the causes of diarrheal disease of infants, recognized pathogenic agents are frequently not recovered. Recently Taylor, Powell and Wright(1), in a study of infantile diarrhea and vomiting, described a serotype of *E. coli* (D433) which was associated with several outbreaks and which was not encountered in 208 control cases. Taylor and her associates found serotype D433 to be identical with the organism described by Giles and Sangster(2); it belongs to a new group, namely, Kauffmann's Group 0111. Since it seemed that this serotype may be of etiological importance in infantile diarrhea, the following studies were undertaken. (1) An attempt was made to determine whether this particular serotype is present in sporadic cases of infantile diarrhea and in *E. coli* infections (pyelitis, peritonitis, meningitis, etc.) of infants, children and adults. (2) The growth characteristics of this serotype on selective culture media were determined in order to ascertain whether it resembles coliform bacteria, being more or less inhibited, or whether it is similar to certain enteric pathogens which are able to grow profusely. (3) Since it was shown(3) recently that salmonellae may be encountered in the upper respiratory tract of children suffering from gastoenteritis, an observation that suggests the possible air-borne transmission of enteric infection from such patients to other individuals, studies on the presence of serotype D433 in the upper respiratory tract of infants were undertaken. (4) The sensitivity of this serotype to various antibiotics was determined *in vitro*. (5) Aureomycin therapy was evaluated in 4 patients suffering from diarrheal disease associated with this microörganism. (6) The effects of ingestion of this organism were observed in 1 infant.

Material and Methods. Through the courtesy of Dr. Joyce Wright, London, England, 4 strains of *E. coli* and 4 antisera were received. Three strains represent serotype D433; the fourth strain, labelled Beta, represents another type, described by Giles, Sangster and Smith(4) and belongs to Kauffmann's Group 055. Antisera were prepared in rabbits and proved to be identical with the sera supplied by Dr. Wright. The sensitivity of the strains to various antibiotics was determined *in vitro*, using serial dilutions of the antibiotics in infusion broth as well as discs containing the antibiotics on seeded Endo agar. Aureomycin hydrochloride (intravenous) was made available through the courtesy of Dr. B. W. Carey of Lederle Laboratories; pure crystalline chloramphenicol (chloromycetin) was kindly supplied by Dr. J. P. Gray of Parke, Davis and Co., polymyxin B sulfate ("Aerosporin") by Dr. D. S. Searle of Burroughs Wellcome and Co., and terramycin hydrochloride by Dr. Elliott R. Weyer of Charles Pfizer and Co. The other antibiotics were procured on the open market. Antibiotic discs containing various amounts of penicillin, streptomycin, chloromycetin, terramycin, and bacitracin were made available by Difco Laboratories through the courtesy of Mr. H. W. Schoenlein. Tablets containing aureomycin were purchased.

Results. *E. coli* serotype D433 was encountered in 5 infants with non-epidemic diarrheal disease.† All strains were typical of *E. coli* and were agglutinated to full titer by

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1. Taylor, J., Powell, B. W., Wright, J., *Brit. Med. J.*, 1949, v2, 117.

2. Giles, C., and Sangster, G., *J. Hyg.*, 1948, v46, 1.

3. Neter, E., *Am. J. Publ. Health*, 1950, v40, 929.

4. Giles, C., Sangster, G., and Smith, J., *Arch. Disease Childhood*, 1949, v24, 45.

† Since this report was prepared, serotype D 433 was isolated from 3 additional cases and the Beta type from another 3 infants with sporadic diarrheal disease.

D433 antisera. Moreover, one of the strains was sent to Dr. Taylor, who corroborated its identity by means of agglutination and agglutinin-absorption tests. Serotype D433 was not present in 33 additional patients with diarrheal disease, either associated with parenteral infection or of undetermined origin. Neither was it found in cultures of feces and of material from nasopharynx and throat of 23 normal premature infants harboring *E. coli* during the first week of life. *E. coli* isolated from urine, peritoneal exudate, spinal fluid, ear drainage and blood from 28 patients suffering from pyelitis (15 cases) peritonitis (6 cases), meningitis (2 cases), otitis media (3 cases), and septicemia (2 cases) were not of this serological type.

The 5 patients who harbored serotype D433 ranged in age from 5 weeks to 3 months. No other pathogenic bacteria were found. The organism was recovered from the feces of all patients, from the nasopharynx of 2 out of 4 and from the throat of 1 out of 4 cases investigated. It is important to emphasize that 3 of the children had neither history or evidence of vomiting. The fact that *E. coli* D433 was found in the throat and nasopharynx of one of the patients who did not have vomiting extends the observations of Taylor and associates(1) who had recovered this serotype from the nasopharynx of infants with vomiting but had not investigated its possible presence in the throat. It is evident that vomiting *per se* is not the sole reason for the presence of this organism in the upper respiratory tract. These observations, together with the recovery of salmonellae(3) and *S. sonnei* (unpublished data) from the upper respiratory tract of infants and children with diarrheal disease, suggest the possibility that these patients may serve as source for air-borne transmission of enteric disease. Following treatment with aureomycin serotype D433 disappeared within a period of 3 to 4 days in 4 patients. In contrast, this organism persisted in the feces of the fifth patient who was treated with sulfadiazine and penicillin. Data on the *in vitro* effectiveness of aureomycin are presented below.

Serotype D433 may be the predominant, gram-negative, aerobic bacillus in the feces of an infected infant as evidenced by the fact that of the 20 isolated colonies of *E. coli* present on Endo agar seeded with feces of 3 of the patients, all belonged to serotype D433. In the remaining 2 cases *E. coli* D433 represented approximately 50% of the coliform organisms present. The cultures from the nasopharynx and throat were comprised of approximately 10% serotype D433 and 90% other coliforms.

Regarding the epidemiological aspects of the diarrheal disease of the 5 patients harboring serotype D433, it may be stated that 2 were twins and that 4 developed the disease at 2 hospitals and one at home. So far as we are aware, there was no outbreak of "epidemic diarrhea of the new-born" at either hospital. These patients, then, have to be considered as sporadic cases of diarrheal disease associated with the presence of serotype D433 in the feces and/or upper respiratory tract. In 2 patients the antibody response was determined, but agglutinins against serotype D433 were not found, 7 and 17 days, respectively, after the onset of the disease.

Thus far, *E. coli* serotype D433 has been incriminated as etiological agent of diarrheal disease solely on epidemiological grounds. Ingestion with the formula of approximately 100 million viable organisms of *E. coli* serotype D433 by a 2-months-old infant with multiple congenital defects, including the brain, who did not previously harbor this microörganism in the feces or respiratory tract, resulted within 24 hours in diarrhea and weight loss of 7 ounces. On the following day serotype D433 was demonstrated in small numbers in the throat and in large numbers in the feces, but not in the nasopharynx. Two days later this organism was present in very large numbers in the nasopharynx, throat and feces. The infant then was treated with terramycin (50 mg/kg/24 hr) which resulted in clinical improvement and the disappearance of this organism within 48 hours. Ingestion of a different type of *E. coli* recovered from the feces of this infant prior to its contact with serotype D433 did not cause diarrhea or weight loss.

Schaub(5), studying the cultural characteristics of paracolon bacilli, found that some (Group 1) are inhibited on selective culture

media, whereas others (Groups 2, 3, and 4) are not. The latter groups resemble the pathogenic enteric organisms. It was deemed of interest, then, to determine whether serotype D433 of *E. coli* is inhibited on such culture media. The strains received from Dr. Wright as well as those isolated in this laboratory were seeded on (1) Endo agar, (2) SS agar and (3) desoxycholate citrate agar. Small inocula were used (1:10.000 dilution of an 18 hour broth culture). It was found that all strains were either completely or considerably inhibited on the two selective media as compared to Endo agar. That these selective culture media are inhibitory is also evident from the fact that *E. coli* serotype D433 was isolated from Endo agar 9 times, from SS agar only once, and from desoxycholate citrate agar only twice, although all media were seeded with all specimens in like manner. It is obvious that these selective culture media should not be used exclusively in the examination of material for the presence of serotype D433 and that this possibly pathogenic serotype does not differ from ordinary coliform bacilli in its growth characteristics on selective media.

It is of interest to know whether *E. coli* serotype D433 differs in its sensitivity to various antibiotics from *E. coli* strains tested previously by various investigators. To this end, the 4 strains received from England and the 5 strains isolated in this laboratory were tested for their sensitivity to the more important antibiotics and sulfadiazine. The results are summarized in Table I. It is interesting to note that all strains were inhibited by the newer antibiotics, aureomycin, chloromycetin, terramycin, and polymyxin B, in a concentration of 10 μg per ml. Streptomycin was distinctly less effective. It is important to emphasize that the strains which were inhibited by small concentrations of one antibiotic were not necessarily inhibited by small concentrations of another. Essentially identical results were obtained in tests employing antibiotic discs and tablets. Obviously,

TABLE 1. Growth Inhibitory Effects of Various Antibiotics and Sulfadiazine on Nine Strains of *E. coli* Serotype D 433.

Antibacterial agent conc./ml	No. of strains inhibited after 24 hr at 37°C
Streptomycin, 100 μg	3
25	3
10	1
Aureomycin, 10 μg	9
5	2
3	2
1	0
Chloromycetin, 10 μg	9
5	6
2.5	4
1	0
Polymyxin B, 10 μg	9
5	7
1	2
Terramycin, 10 μg	9
5	2
1	2
Bacitracin, 400 U	9
200	4
100	0
Penicillin, 500 U	0
Sulfadiazine, 12.5 mg	2

several antibiotics offer themselves for treatment of patients harboring this type of *E. coli*. Observations on the effects of one of them, namely, aureomycin, were referred to above. Favorable results were reported with chloromycetin by Rogers, Koegler, and Gerrard(6). Further investigations on the potential value of different antibiotics in such patients are in progress.

Summary and conclusions. A study of the cultural characteristics and sensitivity to antibiotics of *E. coli* serotype D433 and its occurrence in infants and in patients suffering from various *E. coli* infections revealed the following: (1) Serotype D433 was recovered from 5 sporadic cases of infantile diarrhea; it was present in the feces of all, in the nasopharynx of 2 and in the throat of 1 of these patients. The presence of this microörganism in the upper respiratory tract renders air-borne transmission, particularly in nurseries, a distinct possibility. (2) Serotype D433 was found neither in 33 additional patients suffering from diarrheal disease nor in 23 premature normal infants harboring *E. coli* during the first week of life, nor was it recovered from 28 patients suffering from various *E. coli* in-

5. Schaub, I. G., *Bull. Johns Hopkins Hosp.*, 1948, v83, 367.

6. Rogers, K. B., Koegler, S. J., and Gerrard, J., *Brit. Med. J.*, 1949, v2, 1501.

fections of organs other than the intestinal tract. (3) Ingestion of serotype D433 of *E. coli* resulted in diarrhea and weight loss in a 2-months-old infant, whereas a similar exposure to the patient's own strain of *E. coli* failed to do so. Terramycin therapy was followed by clinical improvement and the disappearance of *E. coli* serotype D433. (4) Serotype D433 (4 strains received from England and 5 isolated in this laboratory) were completely or considerably inhibited on SS agar and desoxycholate citrate agar but grew well on Endo agar. Obviously, these selective culture media should not be used exclusively in examinations for the presence of this organism. (5) Serotype D433 (the above-mentioned 9 strains) was found to be highly susceptible to aureomycin, chloromycetin, terramycin, and polymyxin B (10 μg per ml), but less so to streptomycin. None of the strains were susceptible to bacitracin (100 U per ml) and penicillin (500 U per ml). (6) Treatment with aureomycin of 4 patients resulted in prompt disappearance of serotype D433 and clinical improvement; the fifth patient was treated with sulfadiazine and penicillin and continued to excrete this microorganism in the feces.

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Effects of a Water-Miscible Multiple Vitamin Preparation on Tissue Cultures.* (18247)

PAUL GOLDHABER,† RICHARD A. ORMSBEE‡ AND V. EVERETT KINSEY.
(Introduced by F. J. Stare)

From Massachusetts Eye and Ear Infirmary, Boston, Mass., and the Sloan-Kettering Institute for Cancer Research, New York City.

The present study deals with the effects of a commercial water-miscible multiple vitamin preparation, Vi-Penta, on the morphology of mouse fibroblasts and on the beat of mouse heart fragments grown in tissue culture. This investigation was undertaken because of the possible association of the feeding of water-miscible vitamin preparations to premature infants and the development of an ocular condition, retrolental fibroplasia, leading to blindness(4).

Materials and Methods. All experiments were carried out in roller tube cultures following the technique of Gey and Gey(1). The fibroblasts studied were derived either from mouse embryo skin fragments or from heart fragments of 1- to 3-day-old mice. The tissue fragments were placed in a row in glass culture tubes and then covered with a layer of chicken plasma. After the plasma had clotted, a supernatant fluid of the following composition was added: Tyrode's or Gey's solution, 0.6 ml, heated horse serum§, 0.2 ml and chicken embryo extract, 0.2 ml. The tubes were then stoppered and incubated at 37°C in a rotor. Test materials were incorporated into the supernatant by replacing 0.1 ml of the salt solution with 0.1 ml of the material to be tested. The supernatant was changed approximately twice a week. The cultures of heart fragments were examined periodically in order to determine the number of fragments beating.

Results. Morphologic Effect. After 4 days incubation of mouse embryo skin fragments, a striking morphologic difference was observed in the unstained, living cells exposed to a 1:1,000 dilution of Vi-Penta as compared to

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† Present address: Columbia University, School of Dental and Oral Surgery, New York City, N. Y.

‡ Present address: Rocky Mountain Laboratory, National Institutes of Health, Hamilton, Mont.

1. Gey, G. O., and Gey, M. K., *Am. J. Cancer*, 1936, v27, 45.

§ The horse serum was heated at 56°C for 3 hours to decrease the lysis of the plasma clot(2).