Rev. Inst. Med. trop. São Paulo
11(5):358-362, setembro-outubro, 1969

UDC 616.98
616.34-002

ESCHERICHIA COLI SEROGROUP 0115 ISOLATED FROM PATIENTS WITH ENTERITIS. BIOCHEMICAL CHARACTERISTICS AND EXPERIMENTAL PATHOGENICITY

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SUMMARY

Eight strains of *E. coli* serogroup 0115 isolated from patients with enteritis were studied. All strains were capable of causing experimental keratoconjunctivitis in the guinea-pig. Ingestion of one strain by an adult volunteer was followed by a typical dysenteric syndrome, definite signs of an intense inflammatory reaction in the intestinal mucosa, profuse and increasing growth of the ingested strain and a rising titer of serum agglutinins against the O antigen of the strain. In regard to the biochemical characteristics they were similar to those given by the *Escherichia* group, but lactose was late fermented or not fermented, lysine was not descarboxilated and the eight strains were non-mobile. One strain did not produce gas from glucose and other sugars.

INTRODUCTION

The use of the experimental model described by SERÉNY [7] has permited the discovery of several *E. coli* serotypes which share with *Shigella* strains the capacity of causing experimental keratoconjunctivitis in the guinea-pig. Most of these serotypes have been found in association with cases of enteritis, both in children and adults [10].

The purpose of this paper is to present the result of studies on *E. coli* serogroup 0115 strains, which are capable of causing experimental keratoconjunctivitis in the guinea-pig and intestinal infection in man.

The first strain of the series was isolated in 1964 from an adult patient with severe diarrhea and was designated culture 185T-64[9]. As explained further below, culture 185T-64 was first thought to be a new *E. coli* serogroup [10] but later, upon checking with Dr. F. Orskov, International Escherichia Center, Copenhagen, Denmark, all strains of culture 185T-64 were found to belong to *Escherichia coli* serogroup 0115.

MATERIAL AND METHODS

Strains — Eight strains were studied. They were isolated from children and adult patients with acute diarrhea (Table I). In all cases no recognizable pathogens as *Salmonella*, *Shigella* or enteropathogenic *E. coli* were isolated. Some strains were isolated as nearly pure culture from the stool specimes. The strains were kept in tubes of nutrient agar sealed with paraffined corks.

Biochemical tests — These were made as described by CARPENTER et al. [2], the sodium acetate test being performed as described by TRABULSI & EWING [8].

Pathogenicity to the guinea-pig eye — The test was carried out as described pre-

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TABLE I

Source of strains of *E. coli* serogroup 0115 and reported manifestations of patients

Strains	Patients	
	Age (years old)	Manifestations
185T-64	54	Acute diarrhea for about 24 hours: 15 evacuations, abdominal pains, nauseous sensation, illbeing and fever
185T1-64	27	Acute diarrhea: 4 evacuations a day
185T2-65	1	Acute diarrhea: 4 evacuations
185T3-65	5	Acute diarrhea for 2 days; abdominal pains, vomiting
185T4-65	2	Acute diarrhea
185T5-65	2	Acute diarrhea; fever (38,5º)
185T6-66	?	Acute diarrhea; fever

viously [10]. In all cases the infection was confirmed by bacteriological examinations.

Feeding experiment on an adult volunteer — To perform the experiment strain 185T7-66 was selected out of the 8 studied. It had been isolated a few months ago, from a 3 y.o. child suffering from acute diarrhea. It was sensitive to Neomycin and other drugs. Its virulence to the guinea-pig eye was tested before the feeding-experiment. The inoculum was prepared from the growth of the strain on nutrient agar slant incubated for 18 hours at 37ºC. Organisms were harvested in nutrient broth and 2 ml inoculated into 60 ml of chilled pasteurized milk. The inoculum was fed to the volunteer and the number of viable cells present in it was determinated by plate counting. The inoculum contained $3{,}0 \times 10^8$ viable cells.

The test subject was an adult male, 48 y.o., without symptoms or signs of illness as judged by complete clinical examination. Previous to the experiment, a rectosigmoidoscopic examination was performed, feces were collected for culture and a blood sample taken for serum agglutinin determination. Following intake of the inoculum, temperature was measured at 4 hours intervals and symptoms and signs of illness were recorded. The number of stools passed was recorded and fecal samples were taken for microscopical and bacteriological examination. A second rectosigmoidoscopic examination was performed 24 hours after the inoculation and blood sample for serum agglutinin determination taken 15 days later. After treatment dayly fecal samples were collected for bacteriological examination during 3 days.

The feces cultures were made by seeding the specimes in McConkey and SS agar, and the suspected colonies identified by biochemical and serological tests.

RESULTS

Biochemical characteristics — The results of biochemical tests are shown in Table II. The reactions of the 8 strains were homogenous in most of the tests. However there was some strain variation in the fermentation of lactose, salicin, maltose, rhamnose and in ONPG tests. It should be noted that strain 185T-3 did not produce gas in any of the fermented carbohydrates and strain 185T-5 neither fermented lactose nor gave a positive ONPG reaction.

Pathogenicity to the guinea-pig eye — The 8 strains caused keratoconjunctivitis in the guinea-pig, in all aspects identical to that caused by virulent *Shigella* strains. In

TABLE II

Biochemical characteristics of the 8 strains of *E. coli* serogroup 0115

Substract	185T — 185T1 — 185T2 185T4 — 185T6 — 185T7	185T3	185T5
Indol	+	+	+
VM	+	+	+
VP	—	—	—
Citrate (Simmons)	—	—	—
H_2S	—	—	—
Urease	—	—	—
Phenylalanine deaminose	—	—	—
KCN	—	—	—
Gelatin	—	—	—
Malonate	—	—	—
Lysine decarboxylase	—	—	—
Arginine dihydrolase	—	—	—
Ornithine decarboxilase	—	—	—
Motility	—	—	—
Christensen's citrate	+	+	+
Sodium acetate	+	+	+
ONPG	+	+	—
Glucose	AG	A	AG
Lactose	AG/(AG)/(Ag)	(A)	—
Sucrose	—	—	—
Mannitol	AG	A	AG
Dulcitol	AG	A	AG
Salicin	A/Ag/ag	(A)	(Ag)
Adonitol	—	—	—
Inositol	—	—	—
Sorbitol	AG	A	AG
Arabinose	AG	A	AG
Raffinose	—	—	—
Rhamnose	A/(A)/(a)	(a)	(a)
Maltose	AG/(AG)/(ag)	A	AG
Xylose	AG	A	AG
Trehalose	AG	A	AG
Cellobiose	—	—	—
Dextrin	—	—	—
Starch	—	—	—
Nitrate	+	+	+
Oxidase	—	—	—

A = Strong acid
a = Weak acid
G = Gas 1/4 or more than the volume of Durhan tube
g = Gas less than 1/4 the volume of Durhan tube
( ) = Acid and gas after 48 hours

all of the cases the inoculated strain was demonstrated in the ocular secretion in great amounts during the first days of the disease.

Feeding experiment on an adult volunteer — The pre-feeding examination gave the following results: feces culture showed no pathogens, agglutinins against a boiled suspension of strain 185T-7 was not found at the 1/4 dilution of the serum and the rectosigmoidoscopical examination showed a normal mucosa.

Six hours after the inoculation the volunteer became ill and the illness was allowed to take its course for 18 hours. The volunteer was then trated with Neomycin and antispasmodics. Following the treatment, he felt better and only one further liquid evacuation was passed. In the 3 feces culture perform-

ed in the subsequent 3 days, strain 185T-7 was not found.

The predominant symptoms were diarrhea, abdominal cramps, anorexia and weakness. The last two manifestations were more intense in the second day following inoculation. No rise in temperature was recorded.

A total of 9 evacuations were passed in the 18 hours period. With exception of the first evacuation, feces were liquid, mucous, bloody and contained a great number of leukocytes.

Bacteriological examinations of stool specimes collected from the 1st., 2nd., 3rd., 4th., 7th., 8th., and 9th. evacuations, showed a profuse and increasing growth of strain 185T-7 in both media used. In SS medium, the strain grew as a nearly pure culture from the second evacuation on.

The second rectosigmoidoscopic examination showed that the mucosa was congested and edematiated, bleeding easily. The rectosigmoidoscopy did not progress beyond 14 cm.

The serum agglutinin titer against a heated suspension of strain 185T-7 raised to 1:84 at the second determination.

DISCUSSION

As above mentioned, culture 185T-64 was first thought to be a new *E. coli* serogroup [10]. We came upon this conclusion because the strain was not significantly agglutinated by any of the 01 to 0146 *E. coli* sera and Dr. W. H. Ewing (Enteric Unit, CDC, Atlanta, Ga.) had identified is as "*E. coli* O group undeterminated". The different results obtained in the two first laboratories and in the International Escherichia Center are well explained in a recent paper by GLANTZ et al. [4] who observed the same kind of discrepancy. The controversial results were due to a mixup in the International Salmonella in the distribution of the standard *E. coli* serogroup 0115, in 1950. As a consequence, many laboratories, instead of having the standard strain and antiserum for *E. coli* serogroup 0115, have actually another strain only serologically related to the standard *E. coli* 0115.

E. coli serogroup 0115 was originally isolated by WRAMBY, in Sweden from calves with septicemia. Since then, it has been rather common in different animals diseases, specially in septicemias in calves in Europe [5]. GLANTZ et al. [4] also reported infection in cattle by these organisms in the U.S.A., and confirmed experimentally its pathogenicity for colostrum-deprived calves.

The present studies add to our knowledge on the pathogenicity of *E. coli* serogroup 0115. It has been shown that all the strains isolated from man did cause experimental keratoconjunctivitis in the guinea-pig, and their capacity to cause intestinal infection in man seems to have been proved beyond doubt. Following the ingestion of the inoculum the volunteer develop a typical dysenteric syndrome, with a profuse and increasing growth of the ingested strain, a rising titer of serum agglutinins against the O antigens of the strain and definite signs of an intense inflammatory reaction in the intestinal mucosa, as shown by the rectosigmoidoscopic examination and the presence of blood and pus in feces. Furthermore, all strains were isolated from patients with acute enteritis and in studies which are being carried out we have not found these organisms in the feces of more than 200 normal persons so far tested.

However it should be stressed that the present findings cannot be extend to *E. coli* serogroup 0115 as a whole. A comparative study between the strains isolated in our laboratory and the strains isolated from calves has been not carried out. The latter are typical *E. coli* strains [6] and is not impossible that both groups of strains have non-identical O or K antigens as well as different pathogenic properties.

It is worth to mention that the manifestations presented by the volunteer and the capacity of the strains to cause intestinal infection in adults and experimental keratoconjunctivitis in the guinea-pig indicate that their pathogenic properties are different from those of the classic enteropathogenic *E. coli* serotypes responsible for infantile enteritis. It is more probably that they, as well as others *E. coli* strains virulent to the guinea-pig eye, are very close or identical to *Shigella* in regard to pathogenicity. In re-

gard to this, it is significant that Dr. P. CARPENTER (Disentery Reference Laboratory — Colindale, London) found out by cross absorption tests that strains 185 are strong related if not identical to the provisory *Shigella* type 3341-556 [1].

Most of the others *E. coli* serotypes capable of causing experimental keratoconjunctivitis in the guinea-pig also have 0 antigens identical to those of certain *Shigella* serotypes. This is the case with *E. coli* serotypes 032, 0124, 0143 and so on [3].

In regard to the biochemical characteristics, it should be pointed out that different biotypes were found. It is also noteworthy that lactose was late fermented or not fermented and that all the strains were nonmotile and did not descarboxylate lysine. The latter seems to be a common characteristics to all *Escherichia coli* serotypes capable of causing experimental keratoconjunctivitis in the guinea-pig [10].

RESUMO

Escherichia coli *do grupo sorológico* 0115 *isolado de pacientes com enterite. Características bioquímicas e patogenicidade experimental*

Oito amostras do colibacilo 0115, isoladas de pacientes com enterite, foram estudadas. Tôdas causaram ceratoconjuntivite experimental no cobaio. A ingestão de uma amostra por um voluntário adulto foi seguida de síndrome disentérica típica, sinais de reação inflamatória intensa na mucosa intestinal, proliferação ascendente da amostra e aumento do título de anticorpos séricos contra o antígeno O da amostra ingerida. Bioquìmicamente, as oito amostras se comportaram como *Escherichia*, mas a lactose foi fermentada tàrdiamente por 7 amostras, e não fermentada por uma amostra. Nenhuma amostra descarboxilou a lisina e as oito foram imóveis. Uma amostra não produziu gás dos carboidratos fermentados.

ACKNOWLEDGMENTS

We wish to thank Dr. K. P. Carpenter and F. Orskov for the studies performed and for reading this paper and Dr. W. H. Ewing for the serological tests made with the first strain.

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Recebido para publicação em 29/4/1969.