Rev. Inst. Med. trop. São Paulo
7(4):241-246, julho-agôsto, 1965

UDC 616.981
616.34-002

ON TWO NEW ENTEROBACTERIA PATHOGENIC TO THE GUINEA-PIG EYE (CULTURES 185T-64 AND 193T-64)

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SUMMARY

Two new enterobacteria serotypes pathogenic for the guinea-pig eye are described. Both were isolated from adult patients suffering from acute enteritis.

INTRODUCTION

In 1955 and 1957 it was shown by SERÉNY [8, 9], that the inoculation of *Shigella* cultures into guinea-pig eye was followed by a characteristic disease called by him "keratoconjunctivitis shigellosa". Later on, it was demonstrated by a number of Authors that the guinea-pig keratoconjunctivitis could be produced by other enterobacteria organisms, in addition to those classified as *Shigella*.

As far as we know, four *Enterobacteriaceae* serotypes with this hability have been described up to now. These include *E. coli* 0124:B17 [7, 10, 14] PIÉCHAUD et al. "Parashigella" [6] and another two serotypes whose pertinent historical data may be summarized as follows: the first one was isolated by SCHOLTENS in Holland in 1939 and on the basis of studies with the original or additional strains, named Katwijk-type by SEELIGER (apud [11]), *Shigella boydii*, serotype Ca/972 by CEFALU & GULLOTI [1], *E. coli* 028a028c K 73(B18) by EWING et al.[3] and finally *Shigella scholtensii* by STENZEL [11]. The type culture of the second one was isolated by EWING in Italy, in 1944 and in the same way named since then, serotype 147 by EWING [3], serotype 146/46 by CARPENTER (apud [3]), *Shigella* 13 by MANOLOV [5], *Shigella manolowii* by STENZEL [11] and *Parashigella* by SZTURM-RUBINSTEN et al.[12]. In between the cultures of serotype 147 were identified as anaerogenic *E. coli* O group 25 by EWING et al.[3].

This is a report on two new *Enterobacteriaceae* serotypes which cause typical "Keratoconjunctivitis shigellosa" in guinea-pig, both of them isolated from adult patients suffering from acute enteritis.

Source of cultures

Both cultures were isolated from feces of adult patients suffering from enteritis for about 24 hours. The carrier of culture 185T-64 (G. P., fem., age 53) complained of about 15 evacuations, abdominal pains, nauseous sensation, ill-being and fever. Stools received for examination were liquid and contained plenty of mucus. The carrier of culture 193T-64 (A. B., fem., age 64) complained of 18 bloody and mucous emissions, abdominal pains, tenesm, vomiting, ill-being and fever. Her temperature run to 38.5°C and stools received for examination were liquid, bloody and mucous. Feces were plated on MacConkey agar, SS agar, Des-

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oxycholate citrate agar, Brilhant green agar, and Bismuth sulfite agar, the last two after enrichment in tetrathionate broth. Colonies of both cultures were predominant on the 3 first media but were not recovered on the last two. The colony morphology on these media as well as on plain agar plates conforms by and large to the description recorded for *Shigella* organisms.

Biochemical studies

Unless otherwise indicated, the recommendations of EDWARDS & EWING [2] were followed in the preparation of culture media, reagents and recording of the results of the biochemical tests. However, negative carbohydrate media were kept under observation only for two weeks. Motility was investigated in 0.4% nutrient agar and decarboxylase activities by FALKOW's method.

Both cultures were non motile, Methyl-red-positive and Voges-Proskauer-negative. Sodium citrate (Simmons), malonate and tartarate were not utilized, gelatin not liquified, urea not hydrolised, H_2S not produced and Phenyl-alanine not deaminated by any of the two cultures. Neither culture grew in KCN broth and both failed to ferment sucrose, adonitol, inositol, raffinose, cellobiose. Indole, and gas from glucose, were produced and manitol, sorbitol, maltose, arabinose, trehalose, galactose and mannose promptly fermented by both cultures. Xylose was late fermented by the two and rhamnose promptly by culture 193T-64 but late by culture 185T-64. On the other hand lactose, salicin and dulcitol were fermented, and mucate utilized only by culture 185T-64. Lysine was not decarboxylated and Christensen citrat not utilized by any of them. Arginine and ornithine were decarboxylated after 3 days by culture 185T-64 and ornithine in 24 hours by culture 193T-64. Culture 193T-64 did not decarboxylate arginine and both did grow in acetate [15]. Nitrate was reduced to nitrite and the oxidase test [4] was negative in both cultures.

Serological studies

All sera employed were also prepared in accordance to EDWARDS & EWING [2] recommendations, using as antigen the dried bacteria obtained by the ROSCHKA method. With exception of sera (0) 185T-64 and 193T-64 which were prepared for the present investigation, all the remaining sera had been in use in routine work in our laboratory.

Slide agglutination tests performed with both cultures, unheated or heated in a boiling water-bath for 30 minutes, with sera against all *Shigella* serotypes, *E. coli* 025, 028a028c, and 0124, were always negative but for some very slight agglutinations with cultures 185T-64. On the other hand, the 0 sera prepared were specific for each of these two cultures and did not agglutinate significantly any serological type of *Shigella*, *E. coli* 025, 028a028c, 0124 and the two "Parashigella" Saigon strains either before or after heating of antigens for 30 minutes in the boiling water-bath. These results are shown in Table II.

Pathogenicity of cultures 185T-64 and 193T-64 to the guinea-pig eye

All the techniques employed in this study were described elsewere [13]. Both cultures caused a typical keratoconjunctivitis lasting for about two weeks in several guinea-pigs. Plating of the ocular secretion on MacConkey agar was always followed by luxurous growth during the first days of disease. Agglutination tests carried out in blood samples collected before the inoculation and thereafter at regular intervals showed an increasing antibody titer in all animals tested [13, 14].

Local crossed immunity between Shigella *and cultures 185T-64 and 193T-64 keratoconjunctivitis*

To find out whether a previous *Shigella* infection would protect the guinea-pig against a keratoconjunctivitis by cultures 185T-64 and 193T-64 and *vice-versa*, two preliminary experiments were performed. In both of them guinea-pigs recovered a week earlier of keratoconjunctivitis caused by *Shigella boydii* 2 or by one of the two cultures, were

cross-rechallenged in the same eye with a new infection. Virulence of the three cultures was checked in control guinea-pigs in each test.

Results of experiment No. 1 (culture 185T-64 × *Shigella boydii* 2): the 185T-64 keratoconjunctivitis recovered eye presented a slight inflammatory reaction when inoculated with *Shigella boydii* 2. The organism was found in small amounts on the second and third day of observation, but not on the fifth. The inflammatory reaction disappeared around the fifth day. The control animal (virulence of *Shigella boydii* 2) presented a marked keratoconjunctivitis which lasted for 15 days with abundant proliferation of the inoculated organism during the first week. The *Shigella boydii* 2 keratoconjunctivitis healed eye did not show any manifestation when inoculated with culture 185T-64, and the bacteriological examination became negative on the third day of observation, while the control animal (virulence of culture 185T-64) showed intense keratoconjunctivitis with positive bacteriological examination for 9 days.

Results of experiment No. 2 (culture 193T-64 × *Shigella boydii* 2): as shown on Table III these results closely resemble those of experiment No. 1.

COMMENTS

It is evident from the given description, that cultures 185T-64 and 193T-64 to resemble *E. coli* biochemically (Table I) but are closer to *Shigella* with reference to experimental pathogenicity. They do not only cause typical keratoconjunctivitis in guinea-pigs, but also give rise to local protection against infection by *Shigella* inasmuch as a previous *Shigella* infection affords local protection against 185T-64 and 193T-64 keratoconjunctivitis (Table III). Of course more studies are needed on this line, but as far as we can conclude this cross immunity depends upon a factor which seems to be common to *Shigella* and other enterobacteria causing keratoconjunctivitis in guinea-pig [10, 11].

As stated above cultures 185T-64 and 193T-64 are new enterobacteria serotypes pathogenic to the guinea-pig eye. In fact, data on Table I show that these cultures do not have major antigenic relationship either to any *Shigella* serotype, *E. coli* 0124, 028a028c, 025 or to Szturm-Rubinsten Parashigella (Saigon 62-1344). Furthermore we have been informed by Dr. Szturm-Rubinsten that both cultures were not agglutinated by antiserum for the new *Parashigella* serotype recently described by PIÉCHAUD et al.[6].

For the time being, it is not our wish to discuss taxonomic and nomenclature problems regarding cultures 185T-64 and 193T-64. We would rather refer to them by numbers until more data on both strains as well as on the other closely related enterobacteria have been obtained.

TABLE I

Biochemical characteristics of cultures 185T-64 and 193T-64

	185T-64	193T-64
Indole	+	+
Methyl-red	+	+
Voges-Proskauer	—	—
Citrate (Simmons)	—	—
Phenylalanine	—	—
Urease	—	—
K C N	—	—
H_2S (TSI)	—	—
Gelatin (22°C)	—	—
Lysine (Falkow)	—	—
Arginine (Falkow)	+[3]D	—
Ornithine (Falkow)	+[3]D	+
Glucose (gas)	++	++
Lactose	+[6]D	—
Sucrose	—	—
Salicin	+[4]D	—
Mannitol	+	+
Adonitol	—	—
Inositol	—	—
Dulcitol	+[2]D	—
Sorbitol	+	+
Maltose	+	+
Xylose	+[8]D	+[5]D
Raffinose	—	—
Arabinose	+	+
Trehalose	+	+
Galactose	+	+
Cellobiose	—	—
Rhamnose	+[8]	+
Mannose	+	+
Citrate (Christensen)	—	—
Acetate (TRABULSI & EWING, 1962)	+[2]	+
Malonate	—	—
Mucate	+	—
Tartrate	—	—
Nitrate	+	+
Oxidase	—	—

TABLE II

Slide agglutination tests with cultures 185T-64, 193T-64, *E. coli* 025, 028a028c, 0124 and with *Shigella* types

	Cultures	"0" sera								
		185T-64	193T-64	*E. coli* 025	*E. coli* 028a028c	*E. coli* 0124	*Shigella dysenteriae* (1-10)	*Shigella flexneri* (1-6)	*Shigella boydii* (1-15)	*Shigella sonnei* (I-II)
Unheated	185T-64	++++	—	—	—	—	—	—	—	—
	193T-64	—	++++	—	—	—	—	—	—	—
	E. coli 025	+ VS	—	+ S	⊙	⊙	⊙	⊙	⊙	⊙
	E. coli 028a028c	—	—	⊙	+++	⊙	⊙	⊙	⊙	⊙
	E. coli 0124	—	—	⊙	⊙	+++	⊙	⊙	⊙	⊙
	Shigella dysenteriae (1-10)	—	—	⊙	⊙	⊙	++++	⊙	⊙	⊙
	Shigella flexneri (1-6)	—	—	⊙	⊙	⊙	⊙	++++	⊙	⊙
	Shigella boydii (1-15)	—	—	⊙	⊙	⊙	⊙	⊙	++++	⊙
	Shigella sonnei (I and II)	—	—	⊙	⊙	⊙	⊙	⊙	⊙	++++
	"Parashigella" 62-1344	—	—	++S	⊙	⊙	⊙	⊙	⊙	⊙
	"Parashigella" 63-169	—	—	+ S	⊙	⊙	⊙	⊙	⊙	⊙
Heated	185T-64	++++	—	—	—	—	—	—	—	—
	193T-64	—	++++	—	—	—	—	—	—	—
	E. coli 025	+ VS	—	+++	⊙	⊙	⊙	⊙	⊙	⊙
	E. coli 028a028c	—	—	⊙	+++	⊙	⊙	⊙	⊙	⊙
	E. coli 0124	—	—	⊙	⊙	+++	⊙	⊙	⊙	⊙
	Shigella dysenteriae (1-10)	—	—	⊙	⊙	⊙	⊙	⊙	⊙	⊙
	Shigella flexneri (1-6)	—	—	⊙	⊙	⊙	⊙	⊙	⊙	⊙
	Shigella boydii (1-15)	—	—	⊙	⊙	⊙	⊙	⊙	⊙	⊙
	Shigella sonnei (I and II)	—	—	⊙	⊙	⊙	⊙	⊙	⊙	⊙
	"Parashigella" 62-1344	—	—	+++	⊙	⊙	⊙	⊙	⊙	⊙
	"Parashigella" 63-169	—	—	+++	⊙	⊙	⊙	⊙	⊙	⊙

S = slow agglutination
VS = very slow agglutination
⊙ = agglutination test not performed

TABLE III

Crossed local immunity between *Shigella boydii* 2 and culture 193T-64 (Experiment No. 2)

Guinea-pigs	Days of observation									
	1		3		5		7		9	
	K_e	B_e	K_e	B_e	K_e	B_e	K_e	B_e	K_e	B_e
1. Right eye recovered from keratoconjunctivitis by culture 193T-64 and challenged with *Shigella boydii* 2	—	+	++	++	+	+	—	—	—	—
2. Right eye recovered from keratoconjunctivitis by *Shigella boydii* 2 and challanged with culture 193T-64	±	++	—	+	—	—	—	—	—	—
3. Virulence control of culture 193T-64	++	++++	++++	++++	++++	++++	+++	+++	++	++
4. Virulence control of *Shigella boydii* 2	++	++++	++++	++++	++++	++++	+++	+++	++	++

K_e = keratoconjunctivitis
B_e = bacteriological examination

TRABULSI, L. R.; ZULIANI, M. E. & SERRANO, J. A. — On two new enterobacteria pathogenic to the guinea-pig eye (cultures 185T-64 and 193T-64). *Rev. Inst. Med. trop. São Paulo* **7**:241-246, 1965.

RESUMO

Sôbre duas novas enterobactérias patogênicas para o ôlho do cobaio (culturas 185T-64 *e* 193T-64*)*

Foram descritas duas novas enterobactérias, antigênicamente distintas, isoladas de pacientes adultos com enterite aguda, ambas com a capacidade de determinar ceratoconjuntivite experimental no cobaio.

ACKNOWLEDGMENT

We wish to express our gratitude to Dr. S. Szturm-Rubinsten, Institute Pasteur, Paris, for providing us with two Saigon strains (62-1344 and 63-169) and for studying cultures 185T-64 and 193T-64.

REFERENCES

1. CEFALU, M. & GULLOTI, A. — Un nuovo tipi di *Shigella boydii* "Sierotipo provvisorio Ca/792". *Nuovi ann. igiene e microbiol.* **4**:237-242, 1953.

2. EDWARDS, P. R. & EWING, W. H. — *Identification of Enterobacteriaceae.* 2nd ed., Atlanta, Georgia, Burgess, 1962.

3. EWING, W. H.; REAVIS, R. W. & DAVIS, B. R. — Provisional *Shigella* serotypes. *Canad. J. Microbiol.* **4**:89-107, 1958.

4. KOVACS, N. — Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* (London) **187**(4535):703, 1956.

5. MANOLOV, D. G. — A new species of *Bacterium* of the genus *Shigella* organisms. *J. Microb. Epidem. Immun.* **29**-II:1979-1982, 1958.

6. PIÉCHAUD, D.; SZTURM-RUBINSTEN, S. & CHARPAK, M. — Un nouveau serotype de "Parashigella". *Ann. Inst. Pasteur* **108**: 125-129, 1965.

7. RÉDEY, B. & CSIZMAZIA, F. — The detection of unknown enteric pathogens by conjunctival infection of guinea-pigs. *Acta Microb. Acad. Sci. Hung.* **7**:11-18, 1960.

8. SERÉNY, B. — Experimental *Shigella* keratoconjunctivitis. *Acta Microb. Acad. Sci. Hung.* **2**:293-296, 1955.

9. SERÉNY, B. — Experimental keratoconjunctivitis shigellosa. *Acta Microb. Acad. Sci. Hung.* **4**:367-376, 1957.

10. SERÉNY, B. — Biochemical reactions and virulence of *E. coli* 0124:K72 (B17). *Acta Microb. Acad. Sci. Hung.* **10**:11-18, 1963.

11. STENZEL, W. — *Sh. scholtensii* und *Sh. manolovii* — zwei zur Untergruppe D zu stellende provisorische *Shigella* typen. *Zeitschrift. f. Hyg.* **148**:433-444, 1962.

12. SZTURM-RUBINSTEN, S.; PIÉCHAUD, D. & CHARPAK, M. — *Shigella,* Alkalescens-Dispar: souches intermédiaires. *Ann. Inst. Pasteur,* **106**:122-126, 1964.

13. TRABULSI, L. R. — *Cérato-conjuntivite experimental do cobaio, por enterobactérias. Aspectos etiológicos e sorológicos.* Tese de Livre-Docência, São Paulo, 1964.

14. TRABULSI, L. R. — Experimental keratoconjunctivitis of the guinea-pig by enterobacteria. *Rev. Inst. Med. trop. São Paulo* **7**:16-23, 1965.

15. TRABULSI, L. R. & EWING, W. H. — Sodium acetate medium for the differentiation of *Shigella* and *Escherichia coli* cultures. *Pub. Health Lab.* **20**:137-140, 1962.

Recebido para publicação em 22/6/1965.