BIKEN JOURNAL Vol. 10-1, 25-32, 1967

SHORT COMMUNICATION

THE USE OF YOUNG MOUSE VAGINA AS A MODEL FOR EXPERIMENTAL SHIGELLOSIS

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(Received November 28, 1966)

Many investigators have attempted to develop experimental models of shigellosis, in which they could reproduce a typical lesion of human bacillary dysentery on small experimental animals, and using their methods they have attempted to analyse the pathogenesis and immunity of different strains of *Shigella*.

FORMAL *et al.* (1958, 1963, 1964) reported fatal infections of guinea pigs under special conditions and demonstrated acute purulent inflammatory lesions of the colon and ileum. It is desirable that experimental shigellosis should be reproducible under normal physiological conditions in animals. In this respect, the guinea pig eye is accepted as an excellent site for the detection of virulence of *Shigella* organisms, as reported by ZOELLR *et al.* (1924) and MACKEL *et al.* (1961).

As the mucous membrane of the vagina has a similar type of epithelial cell (stratified squamous epithelium) to the cornea, mouse vagina should also provide a site for experimental shigellosis. This communication is on the survival of infected organisms and the histological changes in the vagina produced by a *Shigella* strain which was characterized as a virulent strain using guinea pig cornea.

Young female ddO mice of 3 to 4 weeks age were used throughout the experiments. The age of the mice was especially important because mice of more than 5 weeks of age did not give reliable results, probably due to a decrease in susceptibility of the thickened vaginal epithelium. *Shigella flexneri* 2a KC^+, *Shigella flexneri* 2a KC^- were isolated at the Kannonji Laboratory, the Osaka Microbial Diseases Research Foundation, and kindly given to use after identification of their corneal reactivity. Both *Sh. flexneri* 2a KC^+ and *Sh. flexneri* 4b KC^+ proved to be virulent, whereas neither *Sh. flexneri* 2a KC^- nor *Sh. flexneri* 4b KC^- produced characteristic keratoconjunctivitis. *Sh. flexneri* 3a and *Sh. sonnei* from fecal specimens were freshly isolated in our laboratory.

The bacterial suspension used for challenge was prepared as follows. The frozen stock culture was incubated on agar slants of brain heart infusions at 37°C for 18 hours. Ten ml of brain heart infusion broth inoculated with one loopful from the slant were shaken at 37°C for 3 hours. Then cells were washed and resuspended in diluted (1:10) broth at a concentration of 10^{10} cells/ml. A loopful (0.005 ml) of this suspension was inoculated onto cleaned mouse vagina at a rate of 5×10^7 organisms per mouse. Vaginal mucus and flora were cleaned out by washing the vagina 3 times with physiological saline containing 0.02 percent merthiolate and then with sterile

TABLE 1 *Recovery of bacteria from mouse vagina 2 days after inoculation*

Strain used	No. of mice tested	Grade of recovery from vagina				
		0	+	++	+++	++++
Sh. flexneri 2a KC$^+$	35			5	19	11
Sh. flexneri 2a KC$^-$	35	18	17			
Sh. flexneri 4b KC$^+$	9				4	5
Sh. flexneri 4b KC$^-$	9	7	2			

Figures indicate number of mice from which each grade of recovery was obtained.

TABLE 2 *Recovery of bacteria from mouse vagina 3 days after inoculation*

Strain used	No. of mice tested	Grade of recovery from vagina				
		0	+	++	+++	++++
Sh. flexneri 2a KC$^+$	6				6	
Sh. flexneri 2a KC$^-$	6	4	2			
Sh. flexneri 4b KC$^+$	4		4			
Sh. flexneri 4b KC$^-$	4	4				

Figures indicate numbers of mice from which each grade of rceovery was obtained.

TABLE 3 *Recovery of bacteria from mouse vagina 2 days after inoculation*

Strain used	No. of mice tested	Grade of recovery from vagina				
		0	+	++	+++	++++
Sh. flexneri 3a-1	5				2	3
Sh. flexneri 3a-2	5			2	3	
Sh. sonnei-1 (phase I)	10				1	9
Sh. sonnei-2 (phase II)	4		4			
Sh. sonnei-3	6				2	4
Sh. sonnei-4						
S (D-1 +, D-2 −)	5				4	1
R (D-1 +, D-2 +)	5	1	4			

Figures indicate number of mice from which each grade of recovery was obtained.

0.01 M phosphate buffered saline using a tapering pippet. The buffered saline remaining in the vaginal lumen was removed with sterile cotton wool, and 5×10^7 cells of *Shigella* were inoculated into the vagina using a platinum loop. Two or three days after the challenge, a loopful of the vaginal contents was spread on a BTB agar plate and incubated at 37°C overnight. The organisms recovered from the vagina were then compared by the number of colonies formed on the BTB agar plate. In the results the number of organisms recovered

from the vagina are graded as 0 ~ ⅲ according to the number of colonies formed, as indicated in Fig. 1.

As shown in Table 1 and 2, the contents of the vagina became purulent within 2 days after infection with KC^+ strain and ⅲ colonies were recovered from the purulent exudates. More than 3 days after the challenge, the number of KC^+ organisms in the vagina began to decrease and *Escherichia coli* became dominant due to contamination of the vagina during feeding. The vagina infected with KC^-, on the other hand, did not develop acute inflammation. No KC^- organisms (0 or +) were recovered as colonies within 2 days after infection. For comparison of the recoveries of the KC^+ and KC^- strains from vaginal inoculations, a 2 days incubation period in the vagina gives satisfactory results for the differentiation of these 2 strains which differ on their virulence in causing keratoconjunctivitis. As shown in Table 3, the recovery of *Sh. flexneri* 3a and *Sh. sonnei* phase I, which were freshly isolated from a patient with diarrhea, was of the same degree as that of *Sh. flexneri* 2a KC^+. On the other hand, the recovery of *Sh. sonnei* phase II, which had been freshly isolated from a healthy carrier person, was less than that of KC^- cells. In addition, the infectivities of both the smooth and rough strains of *Sh. sonnei* isolated from a patient were clearly demonstrated in this experiment. *Sh. sonnei* smooth (D−1 +, D−2 −) strain which agglutinates with anti D−1 but not with anti D−2 serum can be recovered from infected vagina at a grade of ⅲ, whereas *Sh. sonnei* rough (D−1 +, D−2 +) which agglutinates with both anti D−1 and anti D−2 serum gave a low grade of recovery.

Macroscopic observation of the vaginal surface after infection with KC^+ strain revealed marked hyperemia and edematous swelling of the vaginal mucous membrane, whereas vagina infected with KC^- strains did not have an inflammatory appearance. Acute inflammatory changes of the vagina after infection with KC^+ strains were confirmed by histological findings which showed desquamation of epithelial cells and ulcer formation with the infiltration of neutrophil leucocytes into the submucosa (Fig. 2).

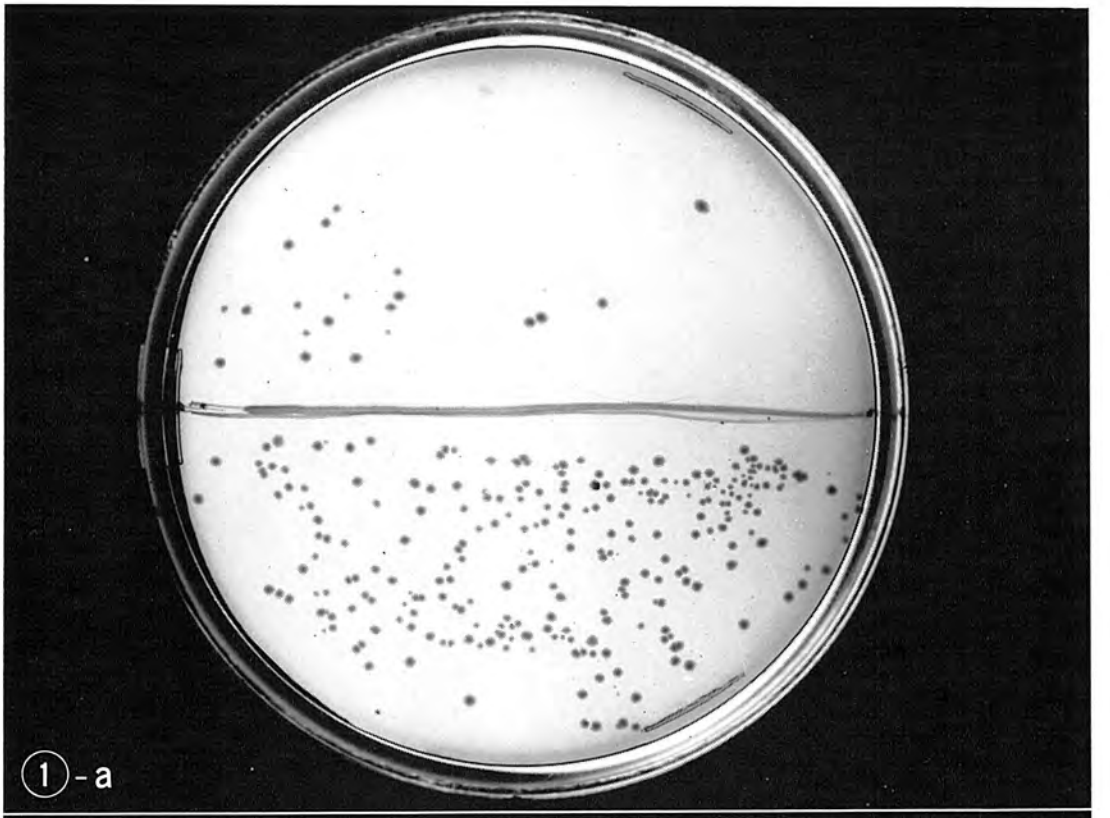
Vagina infected with KC^- strains exhibited less inflammatory change histologically and only edema of the epithelial cells was apparent (Fig. 3). This correlation between recovery of infected organisms from the vagina and acute inflammatory changes induced by KC^+ indicates that the vagina is a convenient site for infection with experimental shigellosis. It is uncertain why KC^- strains are rejected from the vagina so rapidly and why they are incapable of initiating the inflammation, in spite of the fact that the KC^+ and KC^- strains had equal sensitivities to the bactericidal actions of guinea pig alexine and a hydrochloric acid extract of mouse leukocytes.

It may be possible to use the experimental systems described above in developing an effective vaccine for *Shigella*, and work is in progress on this.

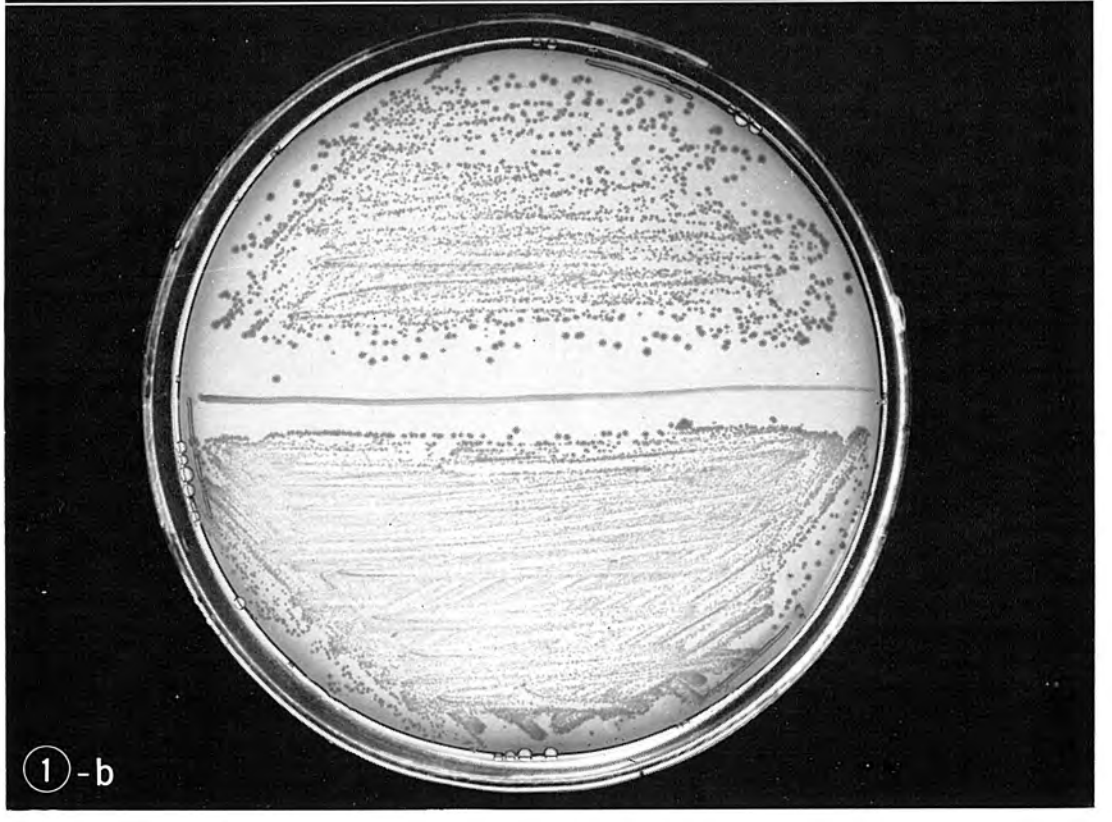
FIGURE 1 Appearance of different grades of colonies of *Shigella* on BTB agar plate.

1-a upper : grade + lower : grade ++

1-b upper : grade +++ lower : grade ++++



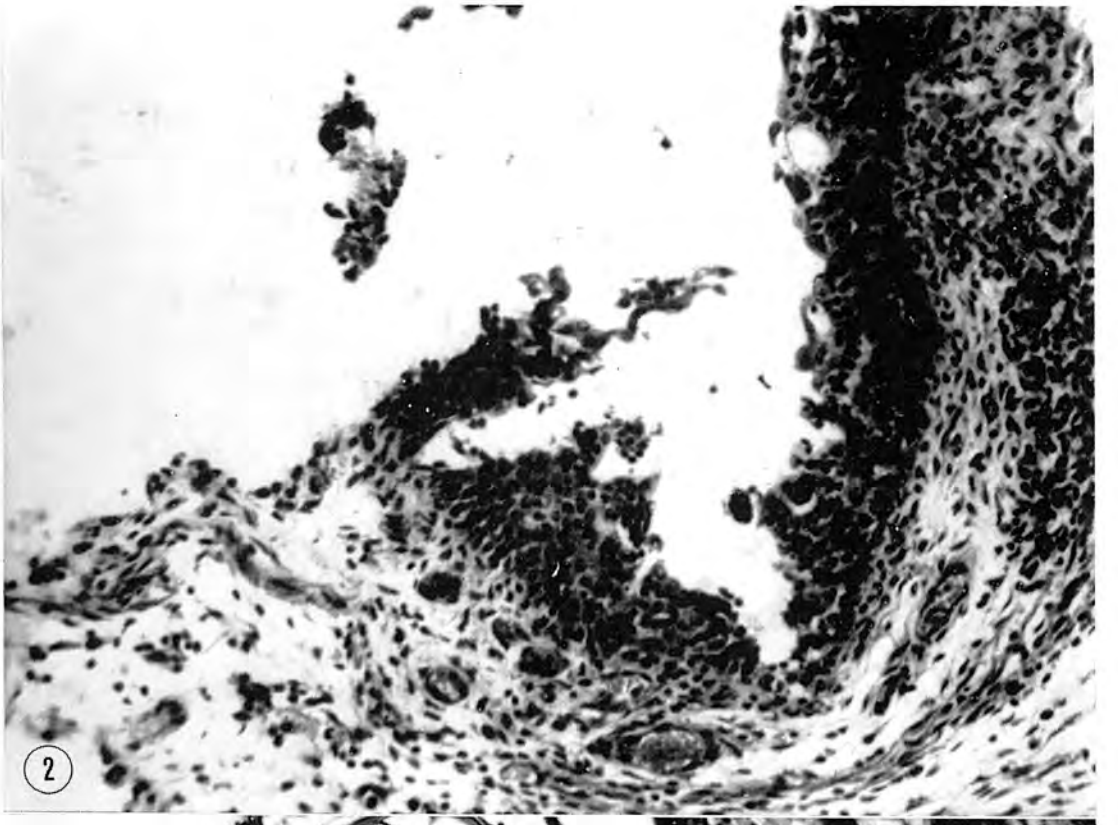
①-a



①-b

FIGURE 2 Section of mouse vagina 3 days after challenge with *Shigella flexneri* 2a KC^+ strain.

FIGURE 3 Section of mouse vagina 3 days after challenge with *Shigella flexneri* 2a KC^- strain.



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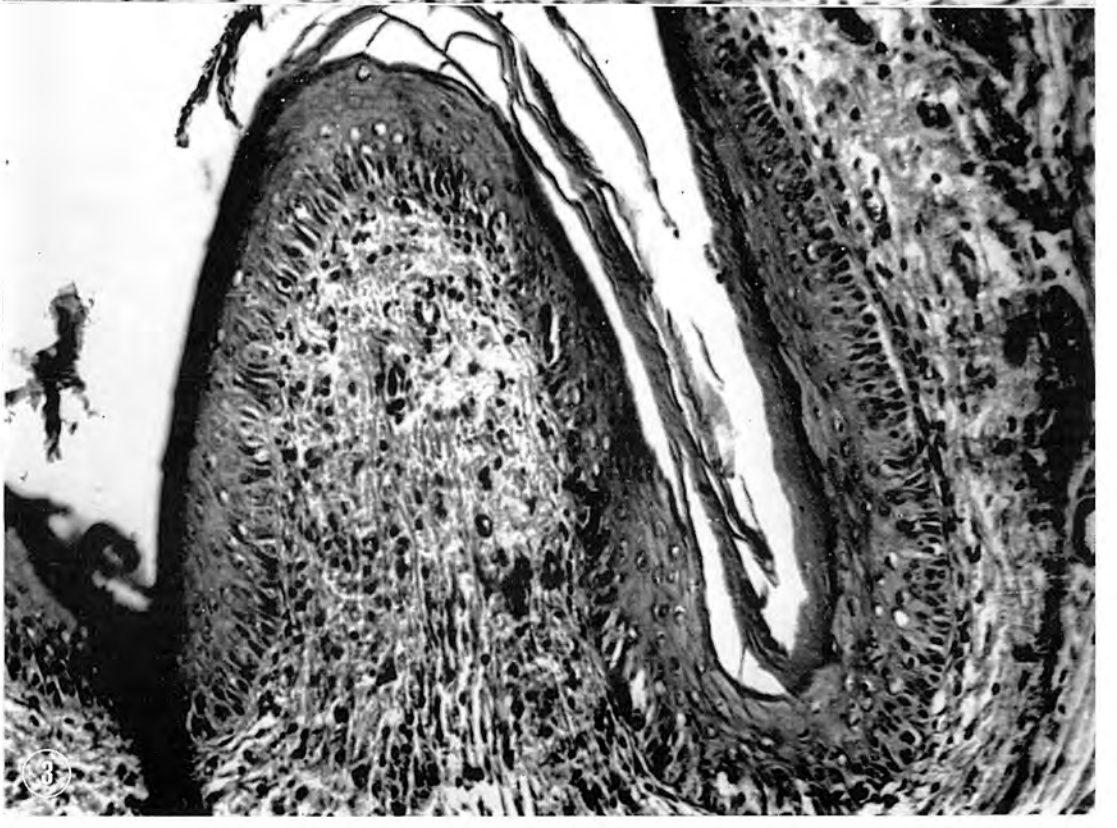

(3)

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